Methods for producing fatty alcohols and derivatives thereof in yeast

Borodina, Irina; Holkenbrink, Carina; Dam, Marie Inger; Löfstedt, Christer

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 June 2018 (21.06.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/109163 A1

(51) International Patent Classification:
C12N 9/02 (2006.01) *C12P 7/04* (2006.01)

(21) International Application Number:
PCT/EP2017/083021

(22) International Filing Date:
15 December 2017 (15.12.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
16204769.0 16 December 2016 (16.12.2016) EP

(71) Applicant: **DANMARKS TEKNISKE UNIVERSITET** [DK/DK]; Anker Engelunds Vej 101 A, 2800 Kgs. Lyngby (DK).

(72) Inventors: **BORODINA, Irina**; Holtebakken 7, 2990 Nivå (DK). **HOLKENBRINK, Carina**; Vermundsgade 18, 2.tv., 2100 Copenhagen Ø (DK). **DAM, Marie, Inger**; Sophie Magdelenes Vej 1B, 2. th., 3460 Birkerød (DK). **LÖFSTEDT, Christer**; Lagerbrings v 5F, 224 60 Lund (SE).

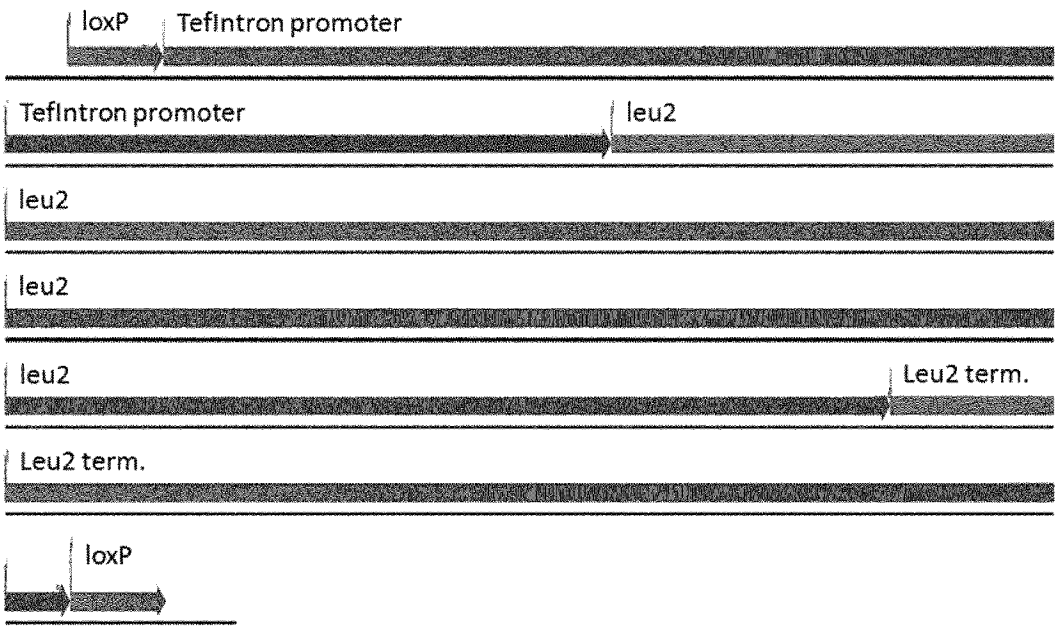
(74) Agent: **HØIBERG P/S**; Adelgade 12, 1304 Copenhagen K (DK).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHODS FOR PRODUCING FATTY ALCOHOLS AND DERIVATIVES THEREOF IN YEAST

FIG. 2



(57) Abstract: The present invention relates to oleaginous yeast cells for the production of fatty alcohols and derivatives thereof, in particular desaturated fatty alcohols, desaturated fatty acyl acetates and desaturated fatty aldehydes. Also provided are methods for obtaining such compounds, which are useful in pheromone compositions.

WO 2018/109163 A1

[Continued on next page]

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

Methods for producing fatty alcohols and derivatives thereof in yeast

Technical field

The present invention relates to oleaginous yeast cells for the production of fatty alcohols and derivatives thereof, in particular desaturated fatty alcohols, desaturated fatty acyl acetates and desaturated fatty aldehydes. Also provided are methods for obtaining such compounds, which are useful in pheromone compositions.

Background

Fatty alcohols are a group of chemicals with broad applications. Fatty alcohols are usually straight primary alcohols that vary in chain length (C_6-C_{26}) and can bear saturated or unsaturated carbon bonds. Depending on the above mentioned characteristics, fatty alcohols can be used as fuel, detergents and surfactants, cosmetics, insect pest management and many more.

Since the advent of DDT more than 50 years ago, broad-spectrum neurotoxic insecticides have provided the principal means for the control of economically important insects in agriculture and public health programs. Whereas the use of synthetic insecticides initially resulted in spectacular increases in crop yields and the suppression of some important human and animal disease vectors, the development of insecticide resistance in insect pest populations and the environmental damage caused by insecticides have become widely recognised as serious drawbacks to their use. Among the most significant environmental problems associated with the manufacture and use of insecticides are 1) their direct toxicity to non-target organisms (including humans); 2) their persistence in the biosphere where they can accumulate and cause untoward developmental and reproductive effects in higher organisms; 3) significant point-source pollution associated with their manufacture and distribution; 4) their worldwide dispersal.

Pheromones can be used as pest control instead of pesticides. (*Z*)19-14:OAc for example has been found to disrupt mating efficiency of fall armyworm with 86% efficiency when applied alone, i.e. without other pheromone components. The commercial use of pheromones to control insect pests by mating disruption has several advantages over conventional insecticides. Pheromones are: 1) non-toxic and

environmentally benign; 2) specific to one target species and do not adversely affect non-target beneficial insects, making them extremely well suited for use in integrated pest management programs; and 3) much less likely (and have never been shown) to produce resistance in the target insect. In contrast to pheromone syntheses in nature, current approaches for the commercial production of pheromones employ traditional synthetic chemical routes. Because pheromones require very high purity to elicit an insect's response, these syntheses are expensive and difficult, and generate large amounts of organic wastes that require treatment.

Thus the major hurdle standing in the way of using sex pheromones remains the production cost. As a result, a very small part of global agricultural land employs pheromones (estimated to less than 0.05%). Pheromone production from a cell factory is expected to significantly lower the production costs of pheromones.

Fatty alcohols are usually produced chemically based on petrochemical or natural feedstocks. Some approaches have been made to produce fatty alcohols in microorganisms such as *Escherichia coli* and yeast using renewable feedstock such as e.g. glucose. Preferable hosts for microbial oleochemical production are oleaginous yeast, which are specialised in accumulating and consuming lipid-derived compounds. Among the oleaginous yeast most advanced research has been conducted on the yeast *Yarrowia lipolytica*.

Methods and host strains are needed to improve the production of fatty alcohols and derivatives thereof.

Summary of invention

The invention is as defined in the claims.

Fatty alcohols can be produced in microorganisms by the heterologous expression of the enzyme fatty acyl reductase which reduces the natively produced fatty acyl-CoA esters to fatty alcohols. In microorganisms fatty acyl-CoA esters are used for membrane lipid production or stored in lipid bodies. Some microorganisms are able to degrade fatty alcohols. *Y. lipolytica* degrades fatty acid derived chemicals via beta-oxidation, which takes place in the peroxisomes, or via omega-oxidation pathway, occurring at the endoplasmic reticulum.

The present disclosure provides methods and host strains which increase fatty alcohol production by decreasing the activity of competing lipid biosynthesis pathway and by decreasing fatty alcohol degradation. These methods and host strains are particularly suitable for production of desaturated fatty alcohols and their derivatives (e.g., fatty acyl acetates), such as Lepidoptera pheromones.

Fatty acyl-CoA esters with different carbon chain length are synthesised by the fatty acyl-CoA synthetase complex (FAS1 and 2) from acetyl-CoA and malonyl-CoA and serve as substrates for storage and membrane lipids. The initial reaction towards lipids is the transfer of fatty acyl-CoAs to glycerol-3-phosphate, catalyzed by glycerol-3-phosphate 1-O-acyltransferase (GPAT) and resulting in formation of lysophosphatidic acid. The overexpression of GPAT in *Y. lipolytica* has been shown to increase lipid accumulation.

The oleaginous yeast *Y. lipolytica* is known to be able to utilize hydrophobic compounds as fatty alcohols as sole energy and carbon source. Fatty alcohols are thought to be oxidised into fatty aldehydes by fatty alcohol dehydrogenases present in both ER and peroxisomes and fatty alcohol oxidases (FAO) present in peroxisomes. The fatty aldehydes are further oxidised to fatty acids by fatty aldehyde dehydrogenases (HFD). *Y. lipolytica*'s Adh1p, Adh3p and Fao1p have been suggested to play a role in degradation of exogenous fatty alcohols, while fatty alcohols resulting from alkane degradation have been suggested to be oxidised by Adh1p-Adh7p, Fadhp and Fao1p (Iwama, 2015). *Y. lipolytica* encodes four fatty aldehyde dehydrogenases, Hfd1p to Hfd4p, which have been shown to be essential for growth on *n*-alkanes (Iwama, 2014). The *PEX10* gene encodes peroxisome biogenesis factor Peroxin 10. Deletion of *PEX10* in *Y. lipolytica* results in the absence of peroxisomes and in defect in *n*-decane assimilation (Sumita et al, 2002).

The present invention describes the downregulation of GPAT, Hfd1, Hfd4, Fao1 and/or Pex10 expression as a strategy to redirect the flux of fatty acyl-CoA ester precursors from storage and membrane lipid synthesis to fatty alcohol production. In combination with the introduction of heterologous desaturases and fatty acyl-CoA reductases, this allows production of fatty alcohols and derivatives thereof with high titres, in particular such fatty alcohols and derivatives which are comprised in *Lepidoptera* pheromones, in an oleaginous yeast cell such as a *Yarrowia lipolytica* cell.

Herein is provided an oleaginous yeast cell capable of producing a desaturated fatty alcohol, said cell:

i) expressing at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) having reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

Also provided is a method of producing a fatty alcohol in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

Also provided is a nucleic acid construct for modifying a yeast cell, said construct comprising:

i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) optionally, additional polynucleotides for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10),

wherein optionally the first polynucleotide and/or the second polynucleotide and/or the additional polynucleotides are under the control of a promoter.

Also provided is a kit of parts comprising:

a) the yeast cell as defined herein and instructions for use; and/or

b) a nucleic acid construct as defined herein, wherein said construct is for modifying a yeast cell, and
c) optionally the yeast cell to be modified.

Also provided is a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the methods disclosed herein.

Also provided is the use of a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the methods disclosed herein.

Description of the drawings

Figure 1: pathway towards Z9-C14:OAc. (1) tetradecanoyl-CoA (myristoyl-CoA), 14:CoA (2) (Z)9-tetradecen-1-yl-CoA, Z9-14:CoA, (3) (Z)9-tetradecen-1-ol, Z9-14:OH, (4) (Z)9-tetradecen-1-yl acetate, Z9-14:OAc, (5) (Z)9-tetradecenal, Z9-14:Ald. Δ9 FAD – Z9-fatty acyl desaturase, FAR – fatty acyl-CoA reductase, AcT – acetyl-CoA transferase.

Figure 2: map of BB1963 (LEU2 selection marker cassette). Leu2 term.: Leu2 terminator.

Figure 3: map of BB1360 (*pex10* and *lip2* terminator). ECS: Easy Cloning Site. pex10 term.: pex10 terminator. lip2 term.: lip2 terminator.

Figure 4: map of expression cassette of Dmd9 and HarFAR. pex20 term: pex20 terminator. TefI prom.: Tefintron promoter. lip2 term.; lip2 terminator.

Figure 5: expression cassette of Atf1. Atf1 YL codon opt: Atf1 codon optimised for *Yarrowia lipolytica*

Figure 6: production of fatty alcohols in various *Y. lipolytica* strains; beyond the genotype indicated on the X axis, the strains also all express the *Amyelois transitella* Δ11 desaturase and the *Heliothis subflexa* reductase (Hs_FAR). Sat.: saturated fatty alcohols (15:OH and 16:OH). Desat.: desaturated fatty alcohols ((*Z*)9-16:OH and (*Z*)11-16:OH).

Detailed description of the invention

Definitions

Biopesticide: the term 'biopesticide' is a contraction of 'biological pesticide' and refers to several types of pest management intervention: through predatory, parasitic, or chemical relationships. In the EU, biopesticides have been defined as "a form of pesticide based on micro-organisms or natural products". In the US, they are defined by the EPA as "including naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs". The present disclosure relates more particularly to biopesticides comprising natural products or naturally occurring substances. They are typically created by growing and concentrating naturally occurring organisms and/or their metabolites including bacteria and other microbes, fungi, nematodes, proteins, etc. They are often considered to be important components of integrated pest management (IPM) programmes, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs). The Manual of Biocontrol Agents (2009: formerly the Biopesticide Manual) gives a review of the available biological insecticide (and other biology-based control) products.

Desaturated: the term "desaturated" will be herein used interchangeably with the term "unsaturated" and refers to a compound containing one or more double or triple carbon-carbon bonds.

Fatty acid: the term "fatty acid" refers to a carboxylic acid having a long aliphatic chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Most naturally occurring fatty acids are unbranched. They can be saturated, or desaturated.

Fatty acyl acetate: the term will herein be used interchangeably with "fatty acetate" and refers to an acetate having a fatty carbon chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty acyl acetates can be saturated or desaturated.

Fatty acyl-CoA: the term will herein be used interchangeably with “fatty acyl-CoA ester”, and refers to compounds of general formula R-CO-SCoA, where R is a fatty carbon chain. The fatty carbon chain is joined to the –SH group of coenzyme A by a thioester bond. Fatty acyl-CoAs can be saturated or desaturated, depending on whether the fatty acid which it is derived from is saturated or desaturated.

Fatty alcohol: the term “fatty alcohol” refers herein to an alcohol derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty alcohols can be saturated or desaturated.

Fatty aldehyde: the term refers herein to an aldehyde derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty aldehydes can be saturated or desaturated.

Heterologous: the term “heterologous” when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is not naturally present in a wild type cell. For example, the term “heterologous Δ9 desaturase” when applied to *Yarrowia lipolytica* refers to a Δ9 desaturase which is not naturally present in a wild type *Y. lipolytica* cell, e.g. a Δ9 desaturase derived from *Drosophila melanogaster*.

Native: the term “native” when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is naturally present in a wild type cell.

Pest: as used herein, the term ‘pest’ shall refer to an organism, in particular an animal, detrimental to humans or human concerns, in particular in the context of agriculture or livestock production. A pest is any living organism which is invasive or prolific, detrimental, troublesome, noxious, destructive, a nuisance to either plants or animals, human or human concerns, livestock, human structures, wild ecosystems etc. The term often overlaps with the related terms vermin, weed, plant and animal parasites and pathogens. It is possible for an organism to be a pest in one setting but beneficial, domesticated or acceptable in another.

Pheromone: pheromones are naturally occurring compounds designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone. Pheromone compositions may be produced chemically or biochemically, for example as described herein. Pheromones may thus comprise desaturated fatty alcohols, fatty aldehydes or fatty acyl acetates, such as can be obtained by the methods and cells described herein.

Reduced activity: the term "reduced activity" may herein refer to a total or a partial loss of activity of a given peptide, such as a protein or an enzyme. In some cases, peptides are encoded by essential genes, which cannot be deleted. In these cases, activity of the peptide can be reduced by methods known in the art, such as down-regulation of transcription or translation, or inhibition of the peptide. In other cases, the peptide is encoded by a non-essential gene, and the activity may be reduced or it may be completely lost, e.g. as a consequence of a deletion of the gene encoding the peptide.

Saturated: the term "saturated" refers to a compound which is devoid of double or triple carbon-carbon bonds.

The present disclosure relates to an oleaginous yeast cell useful for the production of *Lepidoptera* pheromones. The inventors have found that down-regulation of one or more of *HFD1, HFD4, PEX10, FAO1* and *GPAT* in an oleaginous yeast cell, in particular in a *Y. lipolytica* cell, combined with heterologous expression of at least one heterologous desaturase and at least one heterologous fatty acyl-CoA reductase, results in the production of desaturated fatty alcohols useful for example for formulating pheromone compositions for pest management. In particular, down-regulation of *PEX10* and one or more of *HFD1, HFD4, FAO1* or *GPAT* is of interest. The cell may be further engineered to also allow production of fatty alcohol derivatives such as aldehydes and acetates, in particular desaturated fatty alcohol derivatives such as desaturated aldehydes and desaturated acetates, which are also useful for such pheromone compositions.

In one aspect, an oleaginous yeast cell is provided, which is capable of producing a desaturated fatty alcohol, said cell:

i) expressing at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) having reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In some embodiments, the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) has a mutation resulting in reduced activity of Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having a mutation resulting in reduced activity of at least one protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81%

homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

Also provided is a method of producing a fatty alcohol in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of

Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In some embodiments, the method the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) has a mutation resulting in reduced activity of Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having a mutation resulting in reduced activity of at least one protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

Also provided is a nucleic acid construct for modifying a yeast cell, said construct comprising:

i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) optionally, additional polynucleotides for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10),

wherein optionally the first polynucleotide and/or the second polynucleotide and/or the additional polynucleotides are under the control of a promoter.

Also provided is a kit of parts comprising a yeast cell as described herein and instructions for use; and/or a nucleic acid construct as described herein, wherein said construct is for modifying a yeast cell, and optionally a yeast cell to be modified.

Also provided is a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the methods described herein.

Also provided is the use of a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the present methods.

Desaturase (FAD)

In the present disclosure, the terms 'fatty acyl-CoA desaturase', 'desaturase', 'fatty acyl desaturase' and 'FAD' will be used interchangeably. The term refers to an enzyme capable of introducing at least one double bond in E/Z confirmations in an acyl-CoA having a chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 carbon atoms. The double bond may be introduced in any position. For example, a desaturase introducing a double bond in position 3 is termed Δ3 desaturase. A desaturase introducing a double bond in position 5 is termed Δ5 desaturase.A desaturase introducing a double bond in position 6 is termed Δ6 desaturase. A desaturase introducing a double bond in position 7 is termed Δ7 desaturase. A desaturase introducing a double bond in position 8 is termed Δ8 desaturase. A desaturase introducing a double bond in position 9 is termed Δ9 desaturase. A desaturase introducing a double bond in position 10 is termed Δ10 desaturase. A desaturase

introducing a double bond in position 11 is termed Δ11 desaturase. A desaturase introducing a double bond in position 12 is termed Δ12 desaturase. A desaturase introducing a double bond in position 13 is termed Δ13 desaturase. A desaturase introducing a double bond in position 14 is termed Δ14 desaturase. A desaturase introducing a double bond in position 15 is termed Δ15 desaturase. A desaturase introducing a double bond in position 16 is termed Δ16 desaturase. A desaturase introducing a double bond in position 17 is termed Δ17 desaturase. A desaturase introducing a double bond in position 18 is termed Δ18 desaturase. A desaturase introducing a double bond in position 19 is termed Δ19 desaturase. A desaturase introducing a double bond in position 20 is termed Δ20 desaturase.

Reductases reduce acyl-CoAs into alcohols of the corresponding chain length.

In one embodiment, the cell is capable of expressing at least one heterologous Δ5 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ6 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ7 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ8 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ9 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ10 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ11 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ12 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ13 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ14 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ15 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ16 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ17 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ18 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ19 desaturase. In another embodiment, the cell is capable of expressing at least one heterologous Δ20 desaturase.

The skilled person will know, depending on which desaturated fatty alcohol is desired, which kind of desaturase to use. For example, for the production of a fatty alcohol

desaturated in position 11, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 or a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20 may be used. Preferably, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2. If a fatty alcohol desaturated in position 9 is desired, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18 may be used. The gene encoding the heterologous desaturase may be codon-optimised for any purpose for the given host cell, e.g. *Yarrowia lipolytica*, as is known in the art. In one embodiment, the nucleic acid encoding the heterologous desaturase is as set forth in SEQ ID NO: 63 or a homologue thereof having at least 60% homology thereto, , such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology to SEQ ID NO: 63.

Accordingly, in one embodiment, the at least one heterologous desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%,

such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2.

In one embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 1, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 1.

In another embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 64, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%,

such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 64.

In another embodiment, the at least one heterologous desaturase is a Δ9 desaturase having at least 60% homology to the Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18.

In one embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 17, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such

as at least 99%, such as 100% homology to the nucleic acid encoding Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 17.

In another embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 63, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 63.

The heterologous desaturase may preferably be derived from an insect, for example from the order of *Lepidoptera*. In one embodiment, the heterologous desaturase is derived from *Amyelois transitella*. In another embodiment, the heterologous desaturase is derived from *Helicoverpa assulta.* In another embodiment, the heterologous desaturase is derived from *Helicoverpa armigera.* In another embodiment, the heterologous desaturase is derived from *Choristoneura rosaceana*. In another embodiment, the heterologous desaturase is derived from *Choristoneura parallela*.

A heterologous desaturase may be expressed from a nucleic acid introduced in the cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid may be codon-optimised for any purpose as is known in the art for the specific yeast cell used.

The yeast cell to be modified may express a native desaturase, which may have a negative impact on the production of desaturated fatty alcohol and/or desaturated fatty acyl acetate. Accordingly, if the yeast cell to be modified expresses such a native desaturase, the cell may be modified so that activity of the native desaturase is reduced or absent.

To ensure lack of activity of a native desaturase, methods known in the art can be employed. The gene encoding the native desaturase may be deleted or partly deleted in order to ensure that the native desaturase is not expressed. Alternatively, the gene may be mutated so that the native desaturase is expressed but lacks activity, e.g. by mutation of the catalytical site of the enzyme. Alternatively, translation of mRNA to an active protein may be prevented by methods such as silencing RNA or siRNA. Alternatively, the yeast cell may be incubated in a medium comprising an inhibitor which inhibits activity of the native desaturase. A compound inhibiting transcription of the gene encoding the native desaturase may also be provided so that transcription is inactivated when said compound is present.

Inactivation of the native desaturase may thus be permanent or long-term, i.e. the modified yeast cell does not exhibit activity of the native desaturase in stable conditions, or it may be transient, i.e. the modified yeast cell may exhibit activity of the native desaturase for periods of time, but this activity can be suppressed for other periods of time.

Alcohol-forming fatty acyl-CoA reductase (EC 1.2.1.84)

The terms ‘alcohol-forming fatty acyl-CoA reductase’, ‘fatty acyl-CoA reductase’ and ‘FAR’ will be used herein interchangeably. The term “heterologous FAR” refers to a FAR which is not naturally expressed by the yeast cell.

FARs catalyse the two-step reaction (figure 1):
acyl-CoA + 2 NADPH <=> CoA + alcohol + 2 NADP(+)
wherein in a first step, the fatty acyl-CoA is reduced to a fatty aldehyde, before the fatty aldehyde is further reduced into a fatty alcohol in a second step. The fatty acyl-CoA may be a desaturated fatty acyl-CoA.

The FARs capable of catalyzing such reaction are alcohol-forming fatty acyl-CoA reductases with an EC number 1.2.1.84. The yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, and at least one heterologous fatty acyl-CoA reductase capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, and has moreover reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10). In some embodiments, the yeast cell expresses at least one heterologous desaturase, at least one heterologous fatty acyl-CoA reductase and has reduced activity of Pex10 and at least one of Hfd1, Hfd4, GPAT and Fao1 or a homologue thereof.

In some embodiments, the at least one heterologous FAR is derived from an organism belonging to the *Lepidoptera* order.

A heterologous fatty acyl-CoA reductase may be expressed from a nucleic acid introduced in the cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid may be codon-optimised for any purpose as is known in the art for the specific yeast cell used.

In one embodiment, the at least one heterologous FAR is capable of converting a Δ3 fatty acyl-CoA into a Δ3 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ5 fatty acyl-CoA into a Δ5 fatty alcohol.

In another embodiment, the at least one heterologous FAR is capable of converting a Δ6 fatty acyl-CoA into a Δ6 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ7 fatty acyl-CoA into a Δ7 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ8 fatty acyl-CoA into a Δ8 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ9 fatty acyl-CoA into a Δ9 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ10 fatty acyl-CoA into a Δ10 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ11 fatty acyl-CoA into a Δ11 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ12 fatty acyl-CoA into a Δ12 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ13 fatty acyl-CoA into a Δ13 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ14 fatty acyl-CoA into a Δ14 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ15 fatty acyl-CoA into a Δ15 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ16 fatty acyl-CoA into a Δ16 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ17 fatty acyl-CoA into a Δ17 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ18 fatty acyl-CoA into a Δ18 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ19 fatty acyl-CoA into a Δ19 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ20 fatty acyl-CoA into a Δ20 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ21 fatty acyl-CoA into a Δ21 fatty alcohol. In another embodiment, the at least one heterologous FAR is capable of converting a Δ22 fatty acyl-CoA into a Δ22 fatty alcohol.

In some embodiments, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera*, as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24. Preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12.

In one embodiment, the FAR is Har_FAR (SEQ ID NO: 14, FAR from *Helicoverpa armigera*) or a variant thereof having at least 75% homology to Har_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Har_FAR (SEQ ID NO: 14).

In another embodiment, the FAR is Has_FAR (SEQ ID NO: 16, FAR from *Helicoverpa assulta*) or a variant thereof having at least 75% homology to Has_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Has_FAR (SEQ ID NO: 16).

In another embodiment, the FAR is Hs_FAR (SEQ ID NO: 12, FAR from *Heliothis subflexa*) or a variant thereof having at least 75% homology to Hs_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Hs_FAR (SEQ ID NO: 12).

In another embodiment, the FAR is Ban_FAR (SEQ ID NO: 24, FAR from *Bicyclus anynana*) or a variant thereof having at least 75% homology to Ban_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,

such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Ban_FAR (SEQ ID NO: 24).

In some embodiments, expression of the desaturase and/or of the FAR can be induced, for example if the genes encoding these enzymes are under the control of inducible promoters, as is known in the art. The yeast cell is incubated under suitable conditions, such as in an appropriate medium and at an appropriate temperature as is known to a person of skill in the art. Suitable media supporting yeast growth are known in the art and include, but are not limited to: undefined, complete media such as YEPD (or YPD, Yeast Extract Peptone Dextrose); defined, complete medium such as SC (Synthetic Complete); defined, drop-out medium such as SD (Synthetic Dextrose) lacking one or more elements such as an amino acid or an inducer; or mineral medium, consisting of salts, vitamins and a carbon source, and others.

Reduced activity of Hfd1, Hfd4, Pex10, Fao1, GPAT or a homologue thereof

Throughout this disclosure, the term “reduced activity” may refer to a partial or a total loss activity. Reduced activity may be achieved by methods known in the art, e.g. by mutation or deletion of the gene encoding the peptide displaying said activity, by repression of transcription using a repressible promoter, by inhibition of the activity or by silencing at the translational level.

Peroxisome biogenesis factor 10 Pex10

In *Yarrowia lipolytica*, *PEX10* (YALI0C01023g, SEQ ID NO: 7) encodes the peroxisome biogenesis factor 10 (SEQ ID NO: 8). It is involved in import of peroxisomal matrix proteins and localizes to the peroxisomal membrane.

The inventors found that reduced activity of Pex10 resulted in increased titre of desaturated fatty alcohols in *Y. lipolytica.*

Accordingly, the oleaginous yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, at least one heterologous fatty acyl-CoA reductase,

capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described above, and has reduced activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Pex10 (SEQ ID NO: 8).

Reduced or total loss of activity of Pex10 or of a protein having at least 60% homology thereto can be obtained by methods known in the art. In one embodiment, reduced activity is total loss of activity. For example, the gene encoding Pex10 or the protein having at least 60% homology thereto can be knocked out, or deleted, or its transcription can be prevented as is known in the art, for example by introducing a repressible promoter upstream of the gene resulting in total inactivation of transcription in the presence of a repressing compound.

In some embodiments, the gene encoding Pex10 or a protein having at least 60% homology thereto is deleted. In one embodiment, the gene is *PEX10* (SEQ ID NO: 7) and the oleaginous yeast cell is *Yarrowia lipolytica*.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase selected from the group consisting of a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 and a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20; and
- at least one heterologous fatty acyl-CoA reductase selected from the group consisting of a FAR having at least 80% homology to the FAR from *Helicoverpa*

armigera as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* as set forth in SEQ ID NO: 14 and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and

- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at

least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88%

homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology,

such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81%

homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Pex10 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8).

In preferred embodiments, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

Fatty aldehyde dehydrogenase Hfd1

In *Yarrowia lipolytica*, *HFD1* (YALI0F23793g, SEQ ID NO: 3) encodes a fatty aldehyde dehydrogenase (SEQ ID NO: 4). It catalyses the oxidation of fatty aldehydes to fatty acids.

The inventors found that reduced activity of Hfd1 resulted in increased titre of desaturated fatty alcohols in oleaginous yeast cells such as *Y. lipolytica.* In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

Accordingly, the oleaginous yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described above, and has reduced activity of Hfd1 or of a protein having at least 60% homology to Hfd1 (SEQ ID NO: 4), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.
Reduced activity of Hfd1or of a protein having at least 60% homology thereto can be obtained by methods known in the art. In one embodiment, reduced activity is total loss of activity. For example, the gene encoding Hfd1 or the protein having at least 60% homology thereto can be knocked out, or deleted, or its transcription can be prevented as is known in the art, for example by introducing a repressible promoter upstream of the gene resulting in total inactivation of transcription in the presence of a repressing compound.

In some embodiments, the gene encoding Hfd1or a protein having at least 60% homology thereto is deleted. In one embodiment, the gene is *HFD1* (SEQ ID NO: 3) and the oleaginous yeast cell is *Yarrowia lipolytica*.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase selected from the group consisting of a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 and a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20; preferably the at least one heterologous desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2 or a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase selected from the group consisting of a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* as set forth in SEQ ID NO: 14 and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8).

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at

least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology,

such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology,

such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Hfd1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 (SEQ ID NO: 4).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

Fatty aldehyde dehydrogenase Hfd4

In *Yarrowia lipolytica*, *HFD4* (YALI0B01298g, SEQ ID NO: 5 encodes a fatty aldehyde dehydrogenase (SEQ ID NO: 6). It catalyses the oxidation of fatty aldehydes to fatty acids.

The inventors found that reduced activity of Hfd4 resulted in increased titre of desaturated fatty alcohols in *Y. lipolytica.* In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

Accordingly, the oleaginous yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described above, and has reduced activity of Hfd4 or of a protein having at least 60% homology to Hfd4 (SEQ ID NO: 6), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

Reduced activity of Hfd4or of a protein having at least 60% homology thereto can be obtained by methods known in the art. In one embodiment, reduced activity is total loss of activity. For example, the gene encoding Hfd4 or the protein having at least 60% homology thereto can be knocked out, or deleted, or its transcription can be prevented as is known in the art, for example by introducing a repressible promoter upstream of the gene resulting in total inactivation of transcription in the presence of a repressing compound.

In some embodiments, the gene encoding Hfd4or a protein having at least 60% homology thereto is deleted. In one embodiment, the gene is *HFD4* (SEQ ID NO: 5) and the oleaginous yeast cell is *Yarrowia lipolytica*.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase selected from the group consisting of a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 and a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20; preferably the at least one heterologous desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2 or a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase selected from the group consisting of a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* as set forth in SEQ ID NO: 14 and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93%

homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and

- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at

least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Hfd4 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 (SEQ ID NO: 6).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In preferred embodiments, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

Fatty alcohol oxidase Fao1

In *Yarrowia lipolytica*, *FAO1* (YALI0B14014g, SEQ ID NO: 21) encodes a fatty alcohol oxidase (SEQ ID NO: 22). Its deletion results in increased accumulation of ω-hydroxy fatty acids.

The inventors found that reduced activity of Fao1 resulted in increased titre of desaturated fatty alcohols in *Y. lipolytica.* In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.
Accordingly, the oleaginous yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described above, and has reduced activity of Fao1 or of a protein having at least 60% homology to Fao1 (SEQ ID NO: 22), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Fao1 (SEQ ID NO: 22).

Reduced activity of Fao1 or of a protein having at least 60% homology thereto can be obtained by methods known in the art. In one embodiment, reduced activity is total loss

of activity. For example, the gene encoding Fao1 or the protein having at least 60% homology thereto can be knocked out, or deleted, or its transcription can be prevented as is known in the art, for example by introducing a repressible promoter upstream of the gene resulting in total inactivation of transcription in the presence of a repressing compound.

In some embodiments, the gene encoding Fao1 or a protein having at least 60% homology thereto is deleted. In one embodiment, the gene is Fao1 (SEQ ID NO: 21) and the oleaginous yeast cell is *Yarrowia lipolytica*.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase selected from the group consisting of a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 and a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20; preferably the at least one heterologous desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2 or a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase selected from the group consisting of a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* as set forth in SEQ ID NO: 14 and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least

75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81%

homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88%

homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and

- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of Fao1 or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 (SEQ ID NO: 22).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In preferred embodiments, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

Glycerol-3-phosphate acyltransferase GPAT

In *Yarrowia lipolytica*, *GPAT* (YALI0C00209g, SEQ ID NO: 9) encodes a glycerol-3-phosphate acyltransferase (SEQ ID NO: 10). GPAT catalyzes the first reaction towards glycerolipids biosynthesis. The gene is essential in *Yarrowia lipolytica.*

The inventors found that reduced activity of Fao1 resulted in increased titre of desaturated fatty alcohols in *Y. lipolytica.* In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

Accordingly, the oleaginous yeast cell of the present disclosure expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA as described above, at least one heterologous fatty acyl-CoA reductase,

capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described above, and has reduced activity of Fao1 or of a protein having at least 60% homology to GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of GPAT (SEQ ID NO: 10).

Reduced activity of GPAT or of a protein having at least 60% homology thereto can be obtained by methods known in the art. In one embodiment, reduced activity is partial loss of activity. For example, the gene encoding GPAT or the protein having at least 60% homology thereto can be mutated or its transcription can be reduced as is known in the art, for example by introducing a repressible promoter upstream of the gene resulting in partial inactivation of transcription in the presence of a repressing compound. If the yeast cell is *Yarrowia lipolytica,* GPAT activity is reduced without deleting the gene.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase selected from the group consisting of a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2, a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18, a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19 and a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20; preferably the at least one heterologous desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2 or a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and

- at least one heterologous fatty acyl-CoA reductase selected from the group consisting of a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14, a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16, a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12 and a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; preferably, the FAR is selected from a FAR having at least 80% homology to the FAR derived from *Helicoverpa armigera* as set forth in SEQ ID NO: 14 and a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In one embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least

75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81%

homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology,

such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In another embodiment, the yeast cell expresses:

- at least one heterologous desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18; and
- at least one heterologous fatty acyl-CoA reductase having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24; and
- has reduced activity or total loss of activity of GPAT or of a protein having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

In preferred embodiments, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

Reduction of activity of two or more of Hfd1, Hfd4, Pex10, Fao1 and GPAT

It may be advantageous to reduce activity of more than one of the above listed proteins. In some embodiments, the yeast cell has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above, and of one or more of Hfd1, Hfd4, Fao1 and GPAT, or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4, to Hfd4 as set forth in SEQ ID NO: 6, to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

Accordingly, in some embodiments the yeast cell has reduced activity of two or more of Hfd1, Hfd4, Pex10, Fao1 and GPAT, or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4, to Hfd4 as set forth in SEQ ID NO: 6, to Pex10 as set forth in SEQ ID NO: 8, to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

In one embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1 and Hfd4 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82%

homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4 or Hfd4 as set forth in SEQ ID NO: 6.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1 and Pex10 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4 or to Pex10 as set forth in SEQ ID NO: 8.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1 and Fao1 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4 or to Fao1 as set forth in SEQ ID NO: 22.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1 and GPAT or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4 or to GPAT as set forth in SEQ ID NO: 10.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4 and Pex10 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 as set forth in SEQ ID NO: 6 or to Pex10 as set forth in SEQ ID NO: 8.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4 and Fao1 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology,

such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 as set forth in SEQ ID NO: 6 or to Fao1 as set forth in SEQ ID NO: 22.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4 and GPAT or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd4 as set forth in SEQ ID NO: 6 or to GPAT as set forth in SEQ ID NO: 10.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Pex10 and Fao1 or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 as set forth in SEQ ID NO: 8 or to Fao1 as set forth in SEQ ID NO: 22.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Pex10 and GPAT or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 as set forth in SEQ ID NO: 8 or to GPAT as set forth in SEQ ID NO: 10.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Fao1 and GPAT or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

In other embodiments the yeast cell has reduced activity of three or more of Hfd1, Hfd4, Pex10, Fao1 and GPAT, or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93%

homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4, to Hfd4 as set forth in SEQ ID NO: 6, to Pex10 as set forth in SEQ ID NO: 8, to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

In one embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Hfd4 and Pex10 or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Hfd4 and Fao1 or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Hfd4 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Pex10 and Fao1 or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Pex10 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4, Pex10 and Fao1 or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4, Pex10 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Pex10, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In other embodiments the yeast cell has reduced activity of four or more of Hfd1, Hfd4, Pex10, Fao1 and GPAT, or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4, to Hfd4 as set forth in SEQ ID NO: 6, to Pex10 as set forth in SEQ ID NO: 8, to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

In one embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and

has reduced activity of Hfd1, Hfd4, Pex10 and Fao1 or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Hfd4, Pex10 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Pex10, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd1, Hfd4, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In another embodiment, the yeast cell expresses at least one heterologous desaturase as described above and at least one fatty acyl-CoA reductase as described above, and has reduced activity of Hfd4, Pex10, Fao1 and GPAT or homologues thereof having at least 65% homology thereto.

In other embodiments the yeast cell has reduced activity of all of Hfd1, Hfd4, Pex10, Fao1 and GPAT, or homologues thereof having at least 65% homology thereto, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Hfd1 as set forth in SEQ ID NO:4, to Hfd4 as set forth in SEQ ID NO: 6, to Pex10 as set forth in SEQ ID NO: 8, to Fao1 as set forth in SEQ ID NO: 22 or to GPAT as set forth in SEQ ID NO: 10.

Acetyltransferase (EC 2.3.1.84)

The term “acetyltransferase” refers to enzymes of EC number 2.3.1.84 and can also be termed “alcohol-O-acetyltransferase” or “AcT”. It acts on aliphatic alcohols, and catalyses the reaction:

Acetyl-CoA + an alcohol <=> CoA + an acetyl ester.

The yeast cell of the present disclosure may express or overexpress an acetyltransferase. The acetyltransferase may be a native acetyltransferase which the cell to be modified is already capable of expressing, or it may be a heterologous acetyltransferase. If the yeast cell expresses a native acetyltransferase, the yeast cell is preferably modified so that expression of the native acetyltransferase is increased. This can be done by methods known in the art, such as but not limited to introduction of additional copies of the nucleic acid encoding the acetyltransferase in the genome or on a vector, modification of the promoter to a constitutive promoter with a high expression level, or to an inducible promoter which upon induction leads to high expression levels. A heterologous acetyltransferase may be expressed from a nucleic acid introduced in the cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid may be codon-optimised for any purpose as is known in the art for the specific yeast cell used.

If the yeast cell does not express a native acetyltransferase, a nucleic acid encoding a heterologous acetyltransferase may be introduced in the cell, either in a genomic location or on a vector, to enable expression of the acetyltransferase. Preferably, the acetyltransferase is expressed at a high level, e.g. by introducing multiple copies of the nucleic acid encoding the acetyltransferase, or by taking advantage of a constitutive promoter with a high expression level, or of an inducible promoter which upon induction leads to high expression levels.

The term “overexpress” thus refers to the overexpression of an acetyltransferase in a yeast cell when compared to a yeast cell which has not been modified to overexpress the acetyltransferase, i.e. the parent strain.

In some embodiments, the acetyltransferase is the AcT of SEQ ID NO: 62 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_Atf1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 62.

In other embodiments, the conversion of at least part of the desaturated fatty alcohols to desaturated fatty acyl acetates is done chemically, as is known to the skilled person. For example, acetyl chloride can be added to the fatty alcohol and the mixture incubated at room temperature after mixing.

Production of pheromone compounds

The yeast cells of the present disclosure can be used for the production of compounds which are naturally comprised within pheromones, in particular *Lepidoptera* pheromones. Such compounds are typically not naturally produced by oleaginous yeast cells.

The pheromone compounds comprise desaturated and saturated fatty alcohols, desaturated and saturated fatty acyl acetates, and desaturated and saturated fatty aldehydes. Preferably, the compounds produced by the cells and methods of the present disclosure are desaturated.

The compounds obtainable by the present methods using the present cells may be naturally occurring pheromone compounds of a number of *Lepidoptera* species. The larvae of many lepidopteran species are major pests in agriculture. Some of the major pests include *Tortricidae*, *Noctuidae*, and *Pyralidae*. The larvae of the *Noctuidae* genus *Spodoptera* (armyworms), *Helicoverpa* (corn earworm), or *Pieris brassicae* can cause extensive damage to certain crops. *Helicoverpa zea* larvae (cotton bollworms or tomato fruitworms) are polyphagous, meaning they eat a variety of crops, including tomatoes and cotton.

In some embodiments, the compounds obtainable by the present methods using the present cells may be naturally occurring pheromone compounds of the *Tortricidae*, *Noctuidae*, and *Pyralidae* genera. For example, the compounds are naturally occurring in *Spodoptera* or *Helicoverpa.* In some embodiments, the compounds are naturally occurring in *Tuta absoluta* (tomato moth), *Lobesia botrana* (grapevine moth), *Ectomyelois ceratoniae* (carob moth) or *Epiphyas postvittana* (lightbrown apple moth).

Production of desaturated fatty alcohols

The yeast cells of the present disclosure can be used for the production of desaturated fatty alcohols. Such compounds are naturally comprised within pheromones, in particular *Lepidoptera* pheromones, as detailed above.

The present oleaginous yeast cell expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10). In specific embodiments, the yeast cell also has reduced or total loss of activity of Pex10 or of a protein having at least 60% homology to Pex10 (SEQ ID NO: 8), as described above.

Accordingly, herein is provided a method for producing a desaturated fatty alcohol in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

The yeast cell, the desaturase, the fatty acyl-CoA reductase, Hfd1, Hfd4, Pex10, Fao1 and GPAT or homologues thereof may be as defined herein elsewhere.

The fatty alcohols produced by such methods may be saturated or desaturated. Generally, desaturated fatty alcohols are most desirable, and it is an object of the

present disclosure to provide methods for obtaining desaturated fatty alcohols and derivatives thereof with a high titre.

The yeast cell of the present disclosure may thus be used for the production of a range of desaturated fatty alcohols, such as:

- (*Z*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 ;
- (*E*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ5 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 ;
- (*E*)-Δ5 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ6 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ6 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ7 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ7 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ8 desaturated fatty alcohols having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ8 desaturated fatty alcohols having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ9 desaturated fatty alcohols having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ9 desaturated fatty alcohols having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ10 desaturated fatty alcohols having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ10 desaturated fatty alcohols having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ11 desaturated fatty alcohols having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (*E*)-Δ11 desaturated fatty alcohols having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ12 desaturated fatty alcohols having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ12 desaturated fatty alcohols having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ13 desaturated fatty alcohols having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (*E*)-Δ13 desaturated fatty alcohols having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

In one embodiment, the fatty alcohol has a chain length of 8. In another embodiment, the fatty alcohol has a chain length of 9. In another embodiment, the fatty alcohol has a chain length of 10. In another embodiment, the fatty alcohol has a chain length of 11. In another embodiment, the fatty alcohol has a chain length of 12. In another embodiment, the fatty alcohol has a chain length of 13. In another embodiment, the fatty alcohol has a chain length of 14. In another embodiment, the fatty alcohol has a chain length of 15. In another embodiment, the fatty alcohol has a chain length of 16. In another embodiment, the fatty alcohol has a chain length of 17. In another embodiment, the fatty alcohol has a chain length of 18. In another embodiment, the fatty alcohol has a chain length of 19. In another embodiment, the fatty alcohol has a chain length of 20. In another embodiment, the fatty alcohol has a chain length of 21. In another embodiment, the fatty alcohol has a chain length of 22.

The yeast cell disclosed herein may thus express a heterologous Δ9 desaturase and a fatty acyl-CoA reductase, and be used to produce fatty alcohols harbouring a desaturation in *Z* conformation at position 9, for example having a carbon chain length of 14 ((*Z*)9-14:OH). This fatty alcohol is a precursor of (*Z*)9-C14:OAc, which is an important component of pheromones derived from various species, for example the fall armyworm *Spodoptera frugiperda*.

In other embodiments, the yeast cell expresses a heterologous Δ11 desaturase and a fatty acyl-CoA reductase, and can be used to produce fatty alcohols harbouring a desaturation in *Z* conformation at position 9. For example, (*Z*)11-C14:OH may be produced, i.e. a fatty alcohol having a carbon chain length of 14 harbouring a desaturation in *Z* conformation at position 9. This fatty alcohol is a precursor of (*Z*)11-

C14:OAc, which is an important component of pheromones derived from various species, for example the European corn borer *Ostrinia nubilalis* and the red-banded leafroller *Argyrotaenia velutinana*.

The yeast cell disclosed herein may thus express a heterologous Δ11 desaturase, a fatty acyl-CoA reductase, and acetyltransferase and be used to produce fatty alcohol acetates harbouring a desaturation in *Z* conformation at position 11, for example having a carbon chain length of 16 ((*Z*)11-16:OH). This fatty alcohol can be oxidized into (*Z*)11-16:Ald, which is an important component of pheromones derived from various species, for example the cotton bollworm *Helicoverpa armigera*.

In other embodiments, the yeast cell expresses a heterologous Δ11 desaturase and a fatty acyl-CoA reductase, and can be used to produce fatty alcohols harbouring a desaturation in *E* conformation at position 9. For example, (*E*)11-C14:OH may be produced, i.e. a fatty alcohol having a carbon chain length of 14 harbouring a desaturation in *E* conformation at position 9. This fatty alcohol is a precursor of (*E*)11-C14:OAc, which is an important component of pheromones derived from various species, for example the lightbrown apple moth *Epiphyas postvittana*.

The desaturated fatty alcohols produced by the present yeast cell may also be desaturated in more than one position. The desaturated fatty alcohols may be desaturated in at least two positions, such as at least three positions, such as four positions.

For example, (*E*)7, (*Z*)9 desaturated fatty alcohols may be produced having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty alcohols may be produced having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, for example 14. (*Z*)9, (*E*)11, (*E*)13 desaturated fatty alcohols may be produced having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

The thus produced desaturated fatty alcohols may be further modified as is known in the art, for example by carbon chain shortening.

In some embodiments, (*E*)7, (*Z*)9 desaturated fatty alcohols may be produced having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In other

embodiments, (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty alcohols may be produced having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In yet another embodiment, (*Z*)9, (*E*)11, (*E*)13 desaturated fatty alcohols may be produced having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

Production of a desaturated fatty acyl acetate

Fatty acyl acetates, in particular desaturated fatty acyl acetates, are also naturally comprised within pheromones, in particular pheromones produced by species belonging to the *Lepidoptera* order. The yeast cells and methods of the present disclosure can also be used to obtain such fatty acyl acetates.

This can be done by introducing a gene encoding an acetyltransferase, as detailed above, or it can be done by chemicals methods, as is known to the skilled person. For example, acetyl chloride can be added to the fatty alcohol and the mixture incubated at room temperature after mixing.

The yeast cell of the present disclosure may optionally express or overexpress an acetyltransferase capable of converting at least part of the desaturated fatty alcohols produced by the cell in desaturated fatty acyl acetates, and may thus be used for the production of a range of desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as:

- (*Z*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 ;
- (*E*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ5 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ5 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ6 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ6 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ7 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (*E*)-Δ7 desaturated fatty acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ8 desaturated fatty acetates having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ8 desaturated fatty acetates having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ9 desaturated fatty acetates having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ9 desaturated fatty acetates having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ10 desaturated fatty acetates having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ10 desaturated fatty acetates having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ11 desaturated fatty acetates having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ11 desaturated fatty acetates having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ12 desaturated fatty acetates having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ12 desaturated fatty acetates having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ13 desaturated fatty acetates having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (*E*)-Δ13 desaturated fatty acetates having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

In one embodiment, the fatty acyl acetate has a chain length of 8. In another embodiment, the fatty acyl acetate has a chain length of 9. In another embodiment, the fatty acyl acetate has a chain length of 10. In another embodiment, the fatty acyl acetate has a chain length of 11. In another embodiment, the fatty acyl acetate has a chain length of 12. In another embodiment, the fatty acyl acetate has a chain length of 13. In another embodiment, the fatty acyl acetate has a chain length of 14. In another embodiment, the fatty acyl acetate has a chain length of 15. In another embodiment, the fatty acyl acetate has a chain length of 16. In another embodiment, the fatty acyl acetate has a chain length of 17. In another embodiment, the fatty acyl acetate has a

chain length of 18. In another embodiment, the fatty acyl acetate has a chain length of 19. In another embodiment, the fatty acyl acetate has a chain length of 20. In another embodiment, the fatty acyl acetate has a chain length of 21. In another embodiment, the fatty acyl acetate has a chain length of 22.

Accordingly, in one embodiment, the yeast cell expresses a heterologous Δ9 desaturase, a heterologous FAR and an acetyltransferase, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to obtain (*Z*)9-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in *Z* conformation at position 9. This fatty acyl acetate is an important component of pheromones derived from various species, for example the fall armyworm *Spodoptera frugiperda*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In other embodiments, the yeast cell expresses a heterologous Δ11 desaturase, a heterologous FAR and an acetyltransferase, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to produce (*Z*)11-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in *Z* conformation at position 9. This fatty acyl acetate is an important component of pheromones derived from various species, for example the European corn borer *Ostrinia nubilalis* and the red-banded leafroller *Argyrotaenia velutinana*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell expresses a heterologous Δ11 desaturase, a heterologous FAR, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ

ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to obtain (*Z*)11-C16:OH. This fatty alcohol can be oxidized into (*Z*)11-C16:Ald, which is an important component of pheromones derived from various species, for example the cotton bollworm *Helicoverpa armigera*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In other embodiments, the yeast cell expresses a heterologous Δ11 desaturase, a heterologous FAR and an acetyltransferase, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to produce (*E*)11-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in *E* conformation at position 9. This fatty acyl acetate is an important component of pheromones derived from various species, for example the lightbrown apple moth *Epiphyas postvittana*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In other embodiments, the yeast cell expresses a heterologous Δ10 desaturase, a heterologous FAR and an acetyltransferase, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to produce (*Z*)10-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in *Z* conformation at position 10. This compound can be shortened to obtain (*Z*)8-C12:OAc, which is an important component of pheromones derived from various species, for example the oriental fruit moth *Grapholita molesta*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

The desaturated fatty acetates produced by the present yeast cell may also be desaturated in more than one position. The desaturated fatty acetates may be desaturated in at least two positions, such as at least three positions, such as four positions.

For example, (*E*)7, (*Z*)9 desaturated fatty acetates may be produced having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty acetates may be produced having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. (*Z*)9, (*E*)11, (*E*)13 desaturated fatty acetates may be produced having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

The thus produced desaturated fatty acetates may be further modified as is known in the art, for example by carbon chain shortening. Thus, (*E*)7, (*Z*)9 desaturated fatty acetates may be produced having a carbon chain length of 12 starting from fatty acetates having a carbon chain length of 14, (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty acetates may be produced having a carbon chain length of 12 starting from fatty acetates having a carbon chain length of 14, and (*Z*)9, (*E*)11, (*E*)13 desaturated fatty acetates may be produced having a carbon chain length of 12 starting from fatty acetates having a carbon chain length of 14.

In some embodiments, the yeast cell expresses a heterologous Δ9 desaturase, a Δ11 desaturase, a heterologous FAR and an acetyltransferase, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), and can be used to produce (*E*)9, (*Z*)11-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in *E* conformation at position 9 and a desaturation in *Z* conformation at position 11. This compound can be shortened to obtain (*E*)7, (*Z*)9:OAc, which is an important component of pheromones derived from various species, for example the grapevine moth *Lobesia botrana*. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

Production of a desaturated fatty aldehyde

While the present disclosure provides methods for producing desaturated fatty alcohols and desaturated fatty acyl acetates, it may be of interest to further convert said fatty alcohols to the corresponding aldehydes. Thus in some embodiments, the method may further comprise the step of converting at least part of the fatty alcohols to fatty aldehydes, thereby producing fatty aldehydes. This can be achieved by chemical methods or by further engineering of the yeast cell.

In some embodiments, the step of converting at least part of the fatty alcohols to the corresponding aldehydes is a step of chemical conversion. The chemical conversion is based on the oxidation of fatty alcohols to the corresponding aldehydes. Methods for performing this conversion are known in the art. Preferred methods are environmentally friendly and minimize the amount of hazardous waste. In some embodiments, the method comprises a Copper(I)/ABNO-catalysed aerobic alcohol oxidation reaction (Steves & Stahl, 2013).

Thus in some embodiments, the chemical conversion may be metal free, avoiding toxic heavy metal based reagents such as manganese oxides, chromium oxides (Jones ox. PDC, PCC) or ruthenium compounds (TPAP, Ley-Griffith ox.). In some embodiments, the conversion does not involve reactions with activated dimethyl sulfoxide such as the Swern oxidation or the Pfitzner-Moffat type. Such reactions may involve the stereotypic formation of traces of intensively smelling organic sulfur compounds such as dimethyl sulfide which can be difficult to remove from the target product.

In some embodiments, the method comprises a Dess-Martin reaction (Yadav et al., 2004, Meyer et al., 1994).

In other embodiments, the chemical conversion comprises the oxidation with sodium hypochlorite under aqueous/organic two phase conditions (Okada et al., 2014; Tamura et al., 2012; Li et al., 2009).

In some embodiments, the chemical oxidation can be performed with 1-chlorobenzotriazole in a medium of methylene chloride containing 25% pyridine (Ferrell and Yao, 1972).

Alternatively, the oxidation of a fatty alcohol to the corresponding fatty aldehyde can be performed enzymatically by alcohol dehydrogenases. The skilled person will know how to carry out enzymatic oxidation. For example, enzymatic oxidation can be carried out by contacting purified enzymes, cell extracts or whole cells, with the fatty alcohol.

The fatty alcohols obtainable by the cells and methods described herein can be further converted in fatty aldehydes by introducing a gene encoding an aldehyde-forming fatty acyl-CoA reductase EC 1.2.1.50 (FAR'). In this way, at least part of the desaturated fatty acyl-CoA can be converted to the corresponding fatty aldehyde by an aldehyde-forming fatty acyl-CoA reductase (FAR'). The enzymes capable of catalyzing this conversion can catalyse a reduction reaction, where the fatty acyl-CoA is reduced to a fatty aldehyde. Such enzymes are aldehyde-forming fatty acyl-CoA reductases, herein also referred to as FAR' or "aldehyde-forming FAR' ", with an EC number 1.2.1.50. They catalyse the following reaction:

Fatty acyl-CoA + NADPH = fatty aldehyde + NADP+ + coenzyme A.

In some embodiments, expression of the aldehyde-forming FAR' can be induced, for example if the genes encoding this enzyme is under the control of inducible promoters, as is known in the art. The yeast cell is incubated under suitable conditions, such as in an appropriate medium and at an appropriate temperature as is known to a person of skill in the art. Suitable media supporting yeast growth are known in the art and include, but are not limited to: undefined, complete media such as YEPD (or YPD, Yeast Extract Peptone Dextrose), defined, complete medium such as SC (Synthetic Complete), or defined, drop-out medium such as SD (Synthetic Dextrose) lacking one or more elements such as an amino acid or an inducer.

Thus, the following desaturated fatty aldehydes can be obtained:

- (*Z*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ3 desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ5 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ5 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;

- (*Z*)-Δ6 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ6 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ7 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ7 desaturated fatty aldehydes having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ8 desaturated fatty aldehydes having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ8 desaturated fatty aldehydes having a carbon chain length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ9 desaturated fatty aldehydes having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ9 desaturated fatty aldehydes having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ10 desaturated fatty aldehydes having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ10 desaturated fatty aldehydes having a carbon chain length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ11 desaturated fatty aldehydes having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ11 desaturated fatty aldehydes having a carbon chain length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ12 desaturated fatty aldehydes having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*E*)-Δ12 desaturated fatty aldehydes having a carbon chain length of 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22;
- (*Z*)-Δ13 desaturated fatty aldehydes having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22; and
- (*E*)-Δ13 desaturated fatty aldehydes having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22.

In one embodiment, the fatty acyl aldehyde has a chain length of 8. In another embodiment, the fatty acyl aldehyde has a chain length of 9. In another embodiment, the fatty acyl aldehyde has a chain length of 10. In another embodiment, the fatty acyl

aldehyde has a chain length of 11. In another embodiment, the fatty acyl aldehyde has a chain length of 12. In another embodiment, the fatty acyl aldehyde has a chain length of 13. In another embodiment, the fatty acyl aldehyde has a chain length of 14. In another embodiment, the fatty acyl aldehyde has a chain length of 15. In another embodiment, the fatty acyl aldehyde has a chain length of 16. In another embodiment, the fatty acyl aldehyde has a chain length of 17. In another embodiment, the fatty acyl aldehyde has a chain length of 18. In another embodiment, the fatty acyl aldehyde has a chain length of 19. In another embodiment, the fatty acyl aldehyde has a chain length of 20. In another embodiment, the fatty acyl aldehyde has a chain length of 21. In another embodiment, the fatty acyl aldehyde has a chain length of 22.

The desaturated fatty aldehydes produced by the present yeast cell may also be desaturated in more than one position. The desaturated fatty aldehydes may be desaturated in at least two positions, such as at least three positions, such as four positions.

For example, (*E*)7, (*Z*)9 desaturated fatty aldehydes may be produced having a carbon chain length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as 14. (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty aldehydes may be produced having a carbon chain length of 14. (*Z*)9, (*E*)11, (*E*)13 desaturated fatty aldehydes may be produced having a carbon chain length of 14, 15, 16, 17, 18, 19, 20, 21 or 22, such as 14.

The thus produced desaturated fatty aldehydes may be further modified as is known in the art, for example by carbon chain shortening. Thus, (*E*)7, (*Z*)9 desaturated fatty aldehydes may be produced having a carbon chain length of 12 starting from fatty aldehydes having a carbon chain length of 14, (*E*)3, (*Z*)8, (*Z*)11 desaturated fatty aldehydes may be produced having a carbon chain length of 12 starting from fatty aldehydes having a carbon chain length of 14, and (*Z*)9, (*E*)11, (*E*)13 desaturated fatty aldehydes may be produced having a carbon chain length of 12 starting from fatty aldehydes having a carbon chain length of 14.

Fatty acyl-CoA

In order for the yeast cell to produce desaturated fatty alcohols and desaturated fatty acyl acetates as described herein, the yeast cell needs fatty acyl-CoAs as a susbtrate. Preferably, the fatty acyl-CoA has a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21 or 22. In one embodiment, the fatty acyl-CoA has a carbon chain length of 8. In another embodiment, the fatty acyl-CoA has a carbon chain length of 9. In another embodiment, the fatty acyl-CoA has a carbon chain length of 10. In another embodiment, the fatty acyl-CoA has a carbon chain length of 11. In another embodiment, the fatty acyl-CoA has a carbon chain length of 12. In another embodiment, the fatty acyl-CoA has a carbon chain length of 13. In another embodiment, the fatty acyl-CoA has a carbon chain length of 14. In another embodiment, the fatty acyl-CoA has a carbon chain length of 15. In another embodiment, the fatty acyl-CoA has a carbon chain length of 16. In another embodiment, the fatty acyl-CoA has a carbon chain length of 17. In another embodiment, the fatty acyl-CoA has a carbon chain length of 18. In another embodiment, the fatty acyl-CoA has a carbon chain length of 19. In another embodiment, the fatty acyl-CoA has a carbon chain length of 20. In another embodiment, the fatty acyl-CoA has a carbon chain length of 21. In another embodiment, the fatty acyl-CoA has a carbon chain length of 22. In some embodiments a mixture of fatty acyl-CoAs having different carbon chain lengths is provided.

Such fatty acyl-CoAs can either be provided in the medium in which the yeast cell is incubated, or the yeast cell may be naturally able to produce such fatty acyl-CoA, or the yeast cell may be engineered in order to produce or to increase production of such fatty acyl-CoAs.

In some embodiments, the yeast cell is not naturally capable of producing a fatty acyl-CoA having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22, or a mixture thereof. The yeast cell may in this case be engineered as is known in the art, for example by the introduction of a heterologous thioesterase. Thus in some embodiments, a nucleic acid encoding a thioesterase is introduced in the yeast cell, on a vector or by genomic integration. The thioesterase gene may be under the control of an inducible promoter, or under the control of a constitutive promoter. The nucleic acid encoding a thioesterase may be codon-optimised for any purpose for the yeast cell, as is known in the art. In particular, the nucleic acid may be codon-optimised for any purpose for a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

In some embodiments, the thioesterase is derived from an organism selected from *Cuphea palustris, Cuphea hookeriana, Cinnamomum camphora,* or from *Escherichia coli*.

Yeast cell

The present disclosure provides a yeast cell which has been modified to produce a desaturated fatty alcohol, and optionally a desaturated fatty acyl acetate. Desaturated fatty alcohols and desaturated fatty acyl acetates are components of pheromones, in particular of moth pheromones. The yeast cell disclosed herein thus provides a platform for environment-friendly moth pheromone production.

In some embodiments, the cell has been modified at the genomic level, e.g. by gene editing in the genome. The cell may also be modified by insertion of at least one nucleic acid construct such as at least one vector. The vector may be designed as is known to the skilled person to either enable integration of nucleic acid sequences in the genome, or to enable expression of a polypeptide encoded by a nucleic acid sequence comprised in the vector without genome integration.

In certain embodiments of the disclosure, yeast or fungi of genera including, but not limited to, *Blakeslea, Candida, Cryptococcus, Cunninghamella, Lipomyces, Mortierella, Mucor, Phycomyces, Pythium, Rhodosporidium, Rhodotorula, Trichosporon*, and *Yarrowia* are employed. In certain particular embodiments, organisms of species that include, but are not limited to, *Blakeslea trispora, Candida pulcherrima, C. revkaufi, C. tropicalis, Cryptococcus curvatus, Cunninghamella echinulata, C. elegans, C. japonica, Lipomyces starkeyi, L. lipoferus, Mortierella alpina, M. isabellina, M. ramanniana, M. vinacea, Mucor circinelloides, Phycomyces blakesleanus, Pythium irregulare, Rhodosporidium toruloides, Rhodotorula glutinis, R. gracilis, R. graminis, R. mucilaginosa, R. pinicola, Trichosporon pullans, T. cutaneum*, and *Yarrowia lipolytica* are used. In a preferred embodiment, the oleaginous yeast cell is a *Yarrowia lipolytica* cell.

The yeast cell to be modified, which will also be referred to as the host cell, may express native enzymes which are of the same class than the enzymes which are necessary for the production of desaturated fatty alcohols and desaturated fatty acyl acetates. In some cases, however, such native enzymes may have a negative impact on the titre of desaturated fatty alcohols and/or desaturated fatty acyl acetates which can be obtained; the native enzymes may thus be inactivated by methods known in the art, such as gene editing. For example, the genes encoding the native enzymes having

a negative impact on the titre may be deleted or mutated so as to lead to total or partial loss of activity of the native enzyme.

The yeast cell of the present disclosure express at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 as described herein, at least one heterologous fatty acyl-CoA reductase capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described herein, and optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate, and has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10). In some embodiments, the yeast cell also expresses an acetyltransferase. In some embodiments, the yeast cell also expresses a thioesterase. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In one embodiment, the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, and is thereby capable of converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol, and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology,

such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In one embodiment, the yeast cell:

i) expresses at least one heterologous Δ3 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24. In specific embodiments, the yeast cell has reduced or total loss of activity of at

least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ5 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ6 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60%

homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ7 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID

NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ8 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ9 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
ii) expresses at least one heterologous fatty acyl-CoA reductase; and
iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In a particular embodiment, the Δ9 desaturase has at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ10 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ11 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In a particular embodiment, the desaturase has at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2. In another particular embodiment, the desaturase has at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19. In another particular embodiment, the desaturase has at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ12 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
ii) expresses at least one heterologous fatty acyl-CoA reductase; and
iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ13 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
ii) expresses at least one heterologous fatty acyl-CoA reductase; and
iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In another embodiment, the yeast cell:

i) expresses at least one heterologous Δ14 desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14. In other embodiments, the FAR has at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16. In other embodiments, the FAR has at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12. In other embodiments, the FAR has at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

Nucleic acids

It will be understood that throughout the present disclosure, the term 'nucleic acid encoding an activity' shall refer to a nucleic acid molecule capable of encoding a peptide, a protein or a fragment thereof having said activity. Such nucleic acid molecules may be open reading frames or genes or fragments thereof. The nucleic acid construct may also be a group of nucleic acid molecules, which together may

encode several peptides, proteins or fragments thereof having an activity of interest. The term 'activity of interest' refers to one of the following activities: a desaturase as described herein, a fatty acyl-CoA reductase, an aldehyde-forming fatty acyl-CoA reductase, a thioesterase and/or an acetyltransferase activity. The nature of the one or more activity of interest will depend on the nature of the desired product one wishes to obtain with the present methods.

In some embodiments of the present methods, each of the nucleic acids encoding each of the present activities, i.e. a desaturase as described herein, a fatty acyl-CoA reductase, an aldehyde-forming fatty acyl coA reductase, a thioesterase and/or an acetyltransferase, may be comprised within the genome of the yeast cell or within a vector comprised within yeast cell.

In some embodiments, each of the nucleic acids encoding each of the present activities may be present in the genome of said yeast cell, either because the nucleic acid encodes a native protein, or because it has been integrated therein by genome engineering or genome editing or by crossing yeast cells of different mating types. Methods for integrating a nucleic acid are well known in the art. Thus in some embodiments the activity of interest is encoded by introduction of a heterologous nucleic acid in the yeast cell. The heterologous nucleic acid encoding said activity may be codon-optimised for any purpose, or may comprise features that can help improve the activity. For example, the heterologous nucleic acid may be modified so as to encode a modified protein. Such modifications include, but are not limited to, the introduction of localisation signals, gain-of-function or loss-of-function mutations, fusion of the protein to a marker or a tag such as fluorescent tag, insertion of an inducible promoter, introduction of modifications conferring increased stability and/or half-life.

The introduction of the heterologous nucleic acid encoding the activity of interest can be performed by methods known in the art. The skilled person will recognise that such methods include, but are not limited to: cloning and homologous recombination-based methods. Cloning methods may involve the design and construction of a plasmid in an organism such as *Escherichia coli*. The plasmid may be an integrative or a non-integrative vector. Cloning-free methods comprise homologous recombination-based methods such as adaptamer-mediated PCR or gap repair. Such methods often result in integration of the heterologous nucleic acid in the genome of the yeast cell.

The nucleic acids encoding the activities of interest may be present in high copy number.

Methods for production of a fatty alcohol, a fatty acyl acetate or a fatty aldehyde

The yeast cells of the present disclosure can be used for the production of a desaturated fatty alcohol and optionally derivatives thereof, such as a desaturated fatty acyl acetate and/or a desaturated fatty aldehyde. Accordingly, also provided herein is a method of producing a fatty alcohol in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or having reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the yeast cell is capable of producing fatty alcohols with a total titre of at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L, wherein the total titre is the sum of the titre of desaturated fatty alcohols and the titre of saturated fatty alcohols.

In some embodiments, the yeast cell is capable of producing desaturated fatty alcohols with a titre of at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L.

In one embodiment, the present methods enable production of fatty alcohols, wherein the desaturated fatty alcohols produced by the cell represent at least 20% of the total fatty alcohols produced by the cell, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90% of the total fatty alcohols produced by the cell.

In a particular embodiment, the cell is capable of producing (*Z*)11-C16:OH with a titre of at least 0.5 mg/L, such as at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L (*Z*)11-C16:OH.

In another embodiment, the (*Z*)11-C16:OH produced by the cell represents at least 20% of the total fatty alcohols produced by the cell, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90% of the total fatty alcohols produced by the cell.

In yet another embodiment, the (*Z*)11-C16:OH produced by the cell represents at least 20% of the total desaturated fatty alcohols produced by the cell, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90% of the total desaturated fatty alcohols produced by the cell.

In preferred embodiments, the fatty alcohol is a fatty alcohol which is naturally found in pheromones produced by species of the *Lepidoptera* order.

The yeast cell may be as described herein. In some embodiments, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

Recovery

It may be desirable to recover the products obtained by the methods disclosed herein. Thus the present methods may comprise a further step of recovering the desaturated fatty alcohol and/or the desaturated fatty acyl acetate and/or desaturated fatty aldehyde produced by the present yeast cells.

In some embodiments, the method comprises a step of recovering the desaturated fatty alcohols. In a particular embodiment, the method comprises a step of recovering the desaturated fatty alcohols having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In other embodiments, the method comprises a step of recovering the fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22. In a particular embodiment, the method comprises a step of recovering the fatty acyl acetates having a carbon chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22.

Methods for recovering the products obtained by the present invention are known in the art and may comprise an extraction with a hydrophobic solvent such as decane, hexane or a vegetable oil.

The recovered products may be modified further, for example desaturated fatty alcohols may be converted to the corresponding desaturated fatty aldehydes as described herein above.

The recovered products, i.e. the desaturated fatty alcohols and/or desaturated fatty acyl acetates, may also be formulated into a pheromone composition. The composition may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. Fatty aldehydes obtained from said desaturated fatty alcohols may also be comprised in such compositions.

Kit

Provided herein is a kit of parts for performing the present methods. The kit of parts may comprise an oleaginous yeast cell "ready to use" as described herein. In one embodiment, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

In another embodiment, the kit of parts comprises a nucleic acid construct encoding the activities of interest to be introduced in the yeast cell. The kit of parts may comprise a nucleic acid construct for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10). The nucleic acid construct may be provided as a plurality of nucleic acid constructs, such as a plurality of vectors, wherein each vector encodes one or several of the desired activities, or wherein each vector allows reduction of the activity of one or more of Hfd1, Hfd4, Pex10, Fao1 and GPAT. In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

The kit of parts may optionally comprise the yeast cell to be modified. In some embodiments, the yeast cell to be modified already has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO:

8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10). In specific embodiments, the yeast cell has reduced or total loss of activity of at least Pex10 or a protein having at least 60% homology to Pex10 (SEQ ID NO: 8 and at least one of Hfd1, Hfd4, Fao1 or GPAT), as described above.

In some embodiments, the kit of parts comprises all of the above.

Pheromone composition

The present disclosure thus provides compounds, in particular desaturated fatty alcohols and fatty acyl acetates, as well as derivatives thereof, and their use. In particular, the compounds obtainable using the present cells and methods are useful as components of pheromone compositions. Such pheromone compositions may be useful for integrated pest management. They can be used as is known in the art for e.g. mating disruption.

The desaturated fatty alcohols and desaturated fatty acyl acetates obtainable by the present methods or using the present yeast cells may be formulated in a pheromone composition.

Such pheromone compositions may be used as integrated pest management products, which can be used in a method of monitoring the presence of pest or in a method of disrupting the mating of pest.

Pheromone compositions as disclosed herein may be used as biopesticides. Such compositions can be sprayed or dispensed on a culture, in a field or in an orchard. They can also, as is known in the art, be soaked e.g. onto a rubber septa, or mixed with other components. This can result in mating disruption, thereby preventing pest reproduction, or it can be used in combination with a trapping device to entrap the pests. Non-limiting examples of pests against which the present pheromone compositions can be used are: cotton bollworm (*Helicoverpa armigera*), striped stemborer (*Chilo suppressalis*), diamond back moth (*Plutella xylostella*), cabbage moth (*Mamestra brassicae*), large cabbage-heart caterpillar (*Crocidolomia binotalis*), European corn stalk borer (*Sesamia nonagrioides*), currant clearwing (*Synanthedon tipuliformis*) and artichoke plume moth (*Platyptilia carduidactylal*). Accordingly, use of

the present compositions on a culture can lead to increased crop yield, with substantially no environmental impact.

The relative amounts of fatty alcohols and fatty acyl acetates in the present pheromone compositions may vary depending on the nature of the crop and/or of the pest to be controlled; geographical variations may also exist. Determining the optimal relative amounts may thus require routine optimisation. The pheromone compositions may also comprise fatty aldehydes.

Examples of compositions used as repellents can be found in Kehat & Dunkelblum, 1993, for *H. armigera*, in Alfaro et al., 2009, for *C. suppressalis* , in Eizaguirre et al., 2002, for *S. nonagrioides*; in Wu et al., 2012, for *P. xylostella*; in Bari et al., 2003, for *P. carduidactyla*

In some embodiments, the pheromone composition may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. For example, suitable carriers or substrate include vegetable oils, refined mineral oils or fractions thereof, rubbers, plastics, silica, diatomaceous earth, wax matrix and cellulose powder.

The pheromone composition may be formulated as is known in the art. For example, it may be in the form of a solution, a gel, a powder. The pheromone composition may be formulated so that it can be easily dispensed, as is known in the art.

Examples

Example 1: Construction of plasmids

All BioBricks were amplified by PCR to obtain the DNA fragments for cloning into vectors and for gene knockouts. The primers are listed in Table 1 and the resulting DNA fragments are listed in Table 2. The PCR products were separated on a 1%-agarose gel containing Safe-Red® (iNtRON Biotechnology). PCR products of the correct size were excised from the gel and purified using the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel).

Table 1: Primers.

Primer name	**Primer sequence, 5'->3'**	**SEQ ID NO:**

PR-141	agaacagcUgaagcttcgtacg	25
PR-142	AGGCCACUAGTGGATCTGATATCAC	26
PR-10851 (Atrd11 expression cassette _fw)	agtgcaggUgacgcagtaggatgtcctgc	27
PR-10853 (Hs_Far expression cassette _fw)	acctgcacUagagaccgggttgg	28
PR-10655 (EpiVecYL _fw)	acccattgcUgtagatatgtcttgtgtgtaagg	29
PR-10656 (EpiVecYL _rev)	atcatgtaaUtagttatgtcacgcttacattc	30
PR-10702 (Δpex10YL_up _fw)	cattgtaactagtcctggaggg	31
PR-10703 (Δpex10YL_up _rev)	acgaagttaUtttgagccgaggcagatttg	32
PR-10704 (Δpex10YL_down _fw)	acgaagttatUtgacgaggtctggatggaag	33
PR-10705 (Δpex10YL_down _rev)	cattgctaagaatccaaactggag	34
PR-10767 (NatMxSynYL-start _rev_new)	tcatggacatggcatagac	35
PR-11047 (NatMxSynYL-end _rev_new)	aataacttcgUatagcatacattatacgaagttatcgagcgtcccaaaacc	36
PR-11110 (E.coli backboneUSER _fw)	atcgcgtgcattcgcggccgcatttaaatcc	37
PR-11111 (E.coli backboneUSER _rev)	tcgcacgcattcgcggccgcaaatttaaataaaatg	38
PR-11138 (Hphsyn _fw)	agcaatgggUaaaaagcctgaactcaccgc	39
PR-11139 (Hphsyn _rev)	attacatgaUtattcctttgccctcggacg	40
PR-11694 (GPAT_up_USER _fw)	CGTGCGAUgcatctaggagctccattcagc	41
PR-11695 (GPAT_down _USER_rev)	CACGCGAUggacgagcagaccacg	42
PR-13494 (Nat-Tcyc-loxP_fw)	agggtacUactttggatgatactgc	43
PR-13549 (loxP-PrTefIntron _fw)	ataacttcgUataatgtatgctatacgaagttatagagaccgggttggcggcgc	44
PR-14269 (UraYL_fw)	atgccctcctacgaggcccg	45
PR-14270 (UraYL_rev)	ctagcagttgatcttctggtag	46
PR-15426 (Δhfd1_up _fw)	CGTGCGAUataagaaaaaaaacag	47

PR-15427 (Δhfd1_up _rev)	AGCTGTTCUactaaccctacttcctc	48
PR-15428 (Δhfd1_down _fw)	AGTGGCCUttttattggtggtgtg	49
PR-15429 (Δhfd1_down _rev)	CACGCGAUgcatagtgcttttcatattc	50
PR-15438 (Δhfd4_up _fw)	CGTGCGAUagtatcgctactgtactaaaattg	51
PR-15439 (Δhfd4_up _rev)	AGCTGTTCUagcggacaagtgtcaatgtt	52
PR-15440 (Δhfd4_down _fw)	AGTGGCCUatgtattttatcagtagtatctc	53
PR-15441 (Δhfd4_down _rev)	CACGCGAUattggataatacatttccta	54
PR-16463 (Δfao1YL_up _fw)	CGTGCGAUTGGGGGAGGATTGCGATGGG	55
PR-16464 (Δfao1YL_up _rev)	AGCTGTTCUTGTCAAGTAATCAAGCTAATGC	56
PR-16465 (Δfao1YL_down _fw)	AGTGGCCUGCAAGAGACGAGTTTAGAAATAG	57
PR-16466 (Δfao1YL_down _rev)	CACGCGAUGTGTTAGTTCCTTGTAGTGTG	58
PR-16696 (GPAT_up _rev)	agctgttcUTACCGCACTTCCGGAACATC	59
PR-16698 (GPAT_100bpPr_down _fw)	agtggccUCCGATACTTGTTTGTGTGAC	60
PR-10714	ttgcttgcgaacctaattcc	71
PR-10766	ataacttcgUataatgtatgctatacgaagttataaggagtttggcgcccg	72
PR-10767	tcatggacatggcatagac	73
PR-11047	aataacttcgUatagcatacattatacgaagttatcgagcgtcccaaaacc	74
PR-13338	ACCTGCACUgttgatgtgtgtttaattc	75
PR-141	agaacagcUgaagcttcgtacg	76
PR-14148	acgtgcaacgctUacgcaactaacatgaatg	77
PR-14149	TGACTTCAACATTATATCGCTCTGA	78
PR-14279	cgtgcgaUagagaccgggtt	101
PR-16618	AGCCTGCGGUTAGTACTGCAAAAAGTGCTG	102
PR-142	AGGCCACUAGTGGATCTGATATCAC	79
PR-14395	cgtgcgaUttgatatggtgtaacaatg	103
PR-14396	aagcgttgcacgUaagcactatcctctgctgcg	104
PR-14397	agtggccUccgagcgtcgacaagcatac	105

PR-14398	cacgcgaUgttagaagcaattggagaag	106
PR-14565	agtggccUGGGGCTGGCGTGTGAAGGAG	80
PR-14566	acgcgaUCAGACCTCTCACACGGCATC	81
PR-14567	TAATACGACTCACTATAGGGC	82
PR-14568	cacgcgaUCCTTGAGACGTTACCAGAGC	83
PR-14589	cgtgcgaUGCGGAGGAGCAATAGACATACGATTTGAC	84
PR-14590	aagcgttgcacgUTCCCCTCCCCACGGTG	85
PR-14591	agtggccUCACCGAGGGATAGGGAACAC	86
PR-14592	acgcgaUTTAACACTGGACCGTACTGC	87
PR-15430	CGTGCGAUggttctatacgatgg	88
PR-15431	AGCTGTTCUtatgatatttttactaacc	89
PR-15432	AGTGGCCUgattggcgttgtgttcaaatg	90
PR-15433	CACGCGAUttgctcattcaccagaaaag	91
PR-15434	CGTGCGAUatgtacaagtatttctattg	92
PR-15435	AGCTGTTCUgaatgataaagagataacag	93
PR-15436	AGTGGCCUtgtggcggaagttgtacacc	94
PR-15437	CACGCGAUggacaacaggccgatagaac	95
PR-15521	cgtgcgaUaaggagtttggcgcccgtt	107
PR-15522	atgacagaUtgctgtagatatgtcttgt	108
PR-15930	acctgcggtUagtactgcaaaaagtgctgg	96
PR-16592	AGTGCAGGUGCCACAATGGTGCCCAACAAGGGTTC	97
PR-16593	CGTGCGAUCTATCGCTTTCGTCCCCAC	98
PR-16594	aaccgcaggUGGTCCTGACCTCTAAG	99
PR-16595	CACGCGAUCTACTCGTAGGACTTCTTCTC	100
PR-18486	AGTGCAGGUGCCACAATGAACGAGATC	109
PR-18487	CGTGCGAUTTAGGGGCCCAG	110
PR-18490	ACCGCAGGCUCCCTACTCTCGAA	111

PR-18489	CACGCGAUTTATCGAGACTTGTCC	112
PR-18499	ATCTGTCAUGCCACAATGGCTCCCA	113
PR-18500	CACGCGAUTTAGTCGTCCTTGGGG	114

Table 2: DNA fragments obtained by PCR using the indicated template and primers.

DNA fragment ID and name	**Description**	**Fw_primer**	**Rv_primer**	**Template DNA**
BB1135	Vector backbone for propagation in *E.coli*	PR-11110 (E.coli backboneUSER _fw)	PR-11111 (E.coli backboneUSER _rev)	pCfB2196 (Stovicek et al., 2015)
BB01005	Hygromycin resistance gene	PR-11138 (Hphsyn _fw)	PR-11139 (Hphsyn _rev)	Hygromycin resistance gene
BB1131	Hs_FAR expression cassette	PR-10853 (Hs_Far expression cassette _fw)	PR-10655 (EpiVecYL _fw)	pCfB3465
BB1132	Part of pCfB3465 vector	PR-10656 (EpiVecYL _rev)	PR-10851 (Atrd11 expression cassette _fw)	pCfB3465
BB1144	Genomic region upstream of *pex10*	PR-10702 (Δpex10YL_up _fw)	PR-10703 (Δpex10YL_up _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1145	Genomic region downstream of *pex10*	PR-10704 (Δpex10YL_down _fw)	PR-10705 (Δpex10YL_down _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1338	Hygromycin resistance marker	PR-141	PR-142	Hygromycin resistance marker
BB1346	Nourseothricin resistance marker	PR-141	PR-142	Nourseothricin resistance marker
BB1347	2/3 Start of	PR-13549	PR-10767	BB1346

	nourseothricin resistance cassette	(loxP-PrTefIntron _fw)	(NatMxSynYL-start _rev_new)	
BB1348	2/3 end of nourseothricin resistance cassette	PR-13494 (Nat-Tcyc-loxP_fw)	PR-11047 (NatMxSynYL-end _rev_new)	BB1346
BB1349	Genomic region upstream of *pex10* fused to 2/3 Start of nourseothricin resistance cassette	PR-10702 (Δpex10YL_up _fw)	PR-10767 (NatMxSynYL-start _rev_new)	BB1144/BB1347
BB1350	Genomic region downstream of *pex10* fused to 2/3 end of nourseothricin resistance cassette	PR-13494 (Nat-Tcyc-loxP_fw)	PR-10705 (Δpex10YL_down _rev)	BB1348/BB1145
BB1427	Ura3 marker cassette	PR-141	PR-142	Ura3 marker cassette
BB1543	Genomic region upstream of *hfd1*	PR-15426 (Δhfd1_up _fw)	PR-15427 (Δhfd1_up _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1544	Genomic region downstream of *hfd1*	PR-15428 (Δhfd1_down _fw)	PR-15429 (Δhfd1_down _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1549	Genomic region upstream of *hfd4*	PR-15438 (Δhfd4_up _fw)	PR-15439 (Δhfd4_up _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1550	Genomic region downstream of *hfd4*	PR-15440 (Δhfd4_down _fw)	PR-15441 (Δhfd4_down _rev)	Genomic DNA *Yarrowia lipolytica* GB20

BB1725	Genomic region upstream of *fao1*	PR-16463 (Δfao1YL_up _fw)	PR-16464 (Δfao1YL_up _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1726	Genomic region downstream of *fao1*	PR-16465 (Δfao1YL_do wn _fw)	PR-16466 (Δfao1YL_down _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1757	Genomic region upstream of *fao1* fused to 2/3 start of Ura3 cassette	PR-16463 (Δfao1YL_up _fw)	PR-14270 (UraYL_rev)	BB1725/BB1427
BB1758	Genomic region downstream of *fao1* fused to 2/3 end of Ura3 cassette	PR-14269 (UraYL_fw)	PR-16466 (Δfao1YL_down _rev),	BB1726/BB1427
BB1782	Genomic region upstream of *pex10*	PR-11694 (GPAT_up_U SER _fw)	PR-16696 (GPAT_up _rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1784	Genomic region downstream of *pex10*	PR-16698 (GPAT_100b pPr_down _fw)	PR-11695 (GPAT_down _USER_rev)	Genomic DNA *Yarrowia lipolytica* GB20
BB1014	NatSyn	PR-10766	PR-10767	pCfB3405
BB1144	Genomic region upstream of *pex10*	PR-10702	PR-10703	Genomic DNA *Yarrowia lipolytica* GB20
BB1145	Genomic region downstream of *pex10*	PR-10704	PR-10705	Genomic DNA *Yarrowia lipolytica* GB20
BB1351	NatSyn	PR-10714	PR-11047	pCfB3405
BB1352	Pex10_up-NatSyn	PR-10702	PR-10767	BB1144/BB1014
BB1353	NatSyn-Pex10_down	PR-10714	PR-10705	BB1351/BB1145

BB1360	*pex20* and *lip2* terminator	PR-14148	PR-14149	Fig. 3
BB1439	Genomic region upstream of intergenic region IntE_1	PR-14395	PR-14397	Genomic DNA *Yarrowia lipolytica* GB20
BB1440	Genomic region downstream of intergenic region IntE_1	PR-14397	PR-14398	Genomic DNA *Yarrowia lipolytica* GB20
BB1471	Genomic region upstream of intergenic region IntC_1	PR-14565	PR-14566	Genomic DNA *Yarrowia lipolytica* GB20
BB1472	Genomic region downstream of intergenic region IntC_1	PR-14567	PR-14568	Genomic DNA *Yarrowia lipolytica* GB20
BB1483	Genomic region upstream of intergenic region IntE_4	PR-14589	PR-14590	Genomic DNA *Yarrowia lipolytica* GB20
BB1484	Genomic region downstream of intergenic region IntE_4	PR-14591	PR-14592	Genomic DNA *Yarrowia lipolytica* GB20
BB1558	EXP promoter	PR-15521	PR-15522	Genomic DNA *Yarrowia lipolytica* GB20
BB1687	GPD promoter fused to Tefintron promoter	PR-13338	PR-15930	pCfB3465
BB1739	Δ11 desaturase *A. segetum*	PR-16592	PR-16593	Seq ID NO: 67

BB1740	FAR *H. armigera*	PR-16594	PR-16595	Seq ID NO: 14
BB1741	Tefintron promoter	PR- 14279	PR-16618	Genomic DNA *Yarrowia lipolytica* GB20
BB1963	Leu2 selection marker cassette	PR-141	PR-142	Fig. 2
BB2601	SliDes11	PR-18499	PR-18500	SEQ ID NO: 65
BB2602	Dmd9	PR-18490	PR-18489	SEQ ID NO: 63
BB2603	ScAft1	PR-18486	PR-18487	SEQ ID NO: 62

The BioBrick BB1135 was treated with the nicking endonuclease Nb.BsmI (New England Biolabs) according to the manufactures instructions and purified using Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel) prior to use. The DNA fragments were assembled by USER-cloning via the following protocol: 1-1.3 µl of each BioBrick (listed in Table 3), 0.5 µl CutSmart® (New England BioLabs), and 0.5 µl USER enzyme (New England Biolabs) were mixed and incubated at 37°C for 25 min and at 25°C for 25 min. The reaction was transformed into chemically competent *E. coli* DHalpha cells and the cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plates were incubated overnight at 37°C and the resulting colonies were screened by colony PCR. The plasmids were purified from overnight *E. coli* cultures and the correct cloning was confirmed by sequencing. The constructed vectors are listed in Table 3.

The parent plasmids pCfB4778, pCfB5219 and pCfB4781 were digested as described in Jensen et al., 2014 before assembly by USER-cloning. The DNA fragments were assembled by USER-cloning via the following protocol: 1-1.3 µl of each BioBrick (listed in Table 8), 0.5 µl CutSmart® (New England BioLabs), and 0.5 µl USER enzyme (New England Biolabs) were mixed and incubated at 37°C for 25 min and at 25°C for 25 min. The reaction was transformed into chemically competent *E. coli* DHalpha cells and the cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plates were incubated overnight at 37°C and the resulting colonies were screened by colony PCR. The plasmids were purified from overnight *E. coli* cultures and the correct cloning was confirmed by sequencing. The constructed vectors are listed in Table 3.

Table 3: Vectors.

Vector name	Selection marker	DNA fragments assembled
pCfB3465	Ura3	
pCfB5110	NatSyn	BB1135, BB1346, BB1543, BB1544
pCfB5113	HphSyn	BB1135, BB1338, BB1549, BB1550
pCfB3516	HphSyn	BB01005, BB1131, BB1132
pCfB5573	HphSyn	BB1135, BB1338, BB1757, BB1758
pCfB5750	Ura3	BB1135, BB1427, BB1782, BB1784
pCfB4158	Leu2	
pCfB4781	NatSyn	BB1135, BB1360, BB1346, BB1471, BB1472
pCfB4778	Ura3	BB1135, BB1360, BB1439, BB1440, BB1427
pCfB5219	HphSyn	BB1135,BB1360,BB1338, BB1483, BB1484
pCfB5574	NatSyn	BB1135,BB1346,BB1725,BB1726
pCfB5929	HphSyn	pCfB5219, BB1687, BB1739, BB1740
pCfB5930	NatSyn	pCfB4781, BB1687, BB1739, BB1740
pCfB6397	Leu2	BB1135,BB1963,BB1782,BB1784
pCfB7235	Ura3	pCfB4778,BB2603,BB1687,BB1740
pCfB7239	HphSyn	pCfB5219, BB2602, BB1741
pCfB7240	HphSyn	pCfB5219, BB1558, BB2601
pCfB7681	HphSyn	BB1135,BB1338,BB1782,BB1784
pCfB7681	HphSyn	BB1135,BB1338,BB1782,BB1784
pCfB7682	Ura3	BB1135,BB1427,BB1725,BB1726
pCfB7725	NatSyn	BB1135,BB1346,BB1782,BB1784
pCfB7869	NatSyn	BB1135,BB1346,BB1549,BB1550

Example 2: Construction of strains

The constructed strains are listed in Tables 4 and 5. Prior to transformation into *Y. lipolytica*, the expression plasmids were linearised with NotI. The linearised plasmid or BioBricks were transformed into *Y. lipolytica* GB 20 (Angerer, 2014) using a lithium-

acetate-based protocol (Chen, 1997). After transformation the cells were recovered in 500 µl YPD medium for 2 hours at 30°C. Positive transformants were selected on synthetic complete (SC) medium (Sigma-Aldrich) lacking either uracil or leucine or on YPD medium containing hygromycin (50 mg/L) or nourseothricin (250 mg/L). The integration of the knockout constructs into the *Y. lipolytica* genome was confirmed by colony PCR.

The Ura3, NatSyn, HphSyn marker cassettes, which were flanked by loxP sites, were removed from strain ST5255 using a Cre recombinase-based system like following. Strain 5255 was transformed with the vector pCfB4158, expressing the Cre recombinase using a lithium-acetate-based protocol (Chen, 1997). After transformation, the cells were recovered in 500 µl YPD medium for 2 hours at 30°C and plated on SC medium without leucine. Colonies were screened for successful marker removal by replica plating on SC medium without uracil and YPD supplemented with either hygromycin or nourseothricin. No cell growth on these plates indicated successful marker removal.

Strain ST6527 expresses Atrd11 and HarFAR from an intergenic region on chromosome C. The gene expression cassettes are the same as in to pCfB5929. Additionally, the open-reading frame of genes *HFD4* (YALI0B01298g) and *HFD1* (YALI0F23793g), as well as nucleotides -1130 to – 100 upstream of the coding sequence of *GPAT* (YALI0C00209g) were deleted. A premature Stop-codon and frame-shift were introduced into open reading frames of *PEX10* (YALI0C01023g) and *FAO1 ((*YALI0B14014g) resulting in non-functional genes.

Strain ST7394 expresses Dmd9, HarFAR and Atf1 as described in pCfB6969 and pCfB7600 from intergenic regions on chromosomes C and D (figure 4). Additionally, the open-reading frame of genes *HFD4, HFD2, HFD3* and *HFD1*, as well as nucleotides -1130 to – 100 upstream of the coding sequence of *GPAT* (YALI0C00209g) were deleted. A premature Stop-codon and frame-shift was introduced into *PEX10* and *FAO1* resulting in non-functional genes.

Table 4: Strains. Atrd11 = Δ11 desaturase from *Amyelois transitella;* HsFAR = fatty acyl-CoA reductase from *Heliothis subflexa*

Strain name	Strain description	Parent strain	Plasmids/BioBricks
ST3683	Yarrowia lipolytica GB20	-	-

ST3737	Δ*pex10*	ST3683	BB1352/BB1353
ST3842	Δ*pex10* Atrd11 Hs_FAR	ST3737	pCfB3516
ST3844	Atrd11 Hs_FAR	ST3683	pCfB3465
ST5107	Atrd11 Hs_FAR Δ*hfd1*	ST3844	pCfB5110
ST5110	Atrd11 Hs_FAR Δ*hfd4*	ST3844	pCfB5113
ST5255	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4*	ST5107	pCfB5113
ST5791	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1* GPAT_100bpPr	ST5789	pCfB5750
ST6277	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1* GPAT_100bpPr	ST5789	pCfB6397
ST6289	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1* GPAT_100bpPr Atrd11 HarFAR	ST6277	pCfB5929
ST6360	Δ*hfd4* Δ*hfd1* Δ*pex10* Δ*fao1* Atrd11 Des11 HsFAR HarFAR Atf1	ST5789	pCfB7235 pCfB7240
ST6379	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1* GPAT_100bpPr Atrd11 HarFAR Atrd11 HarFAR	ST6289	pCfB5930
ST7458	Atrd11 HsFAR 100bp_PrGPAT	ST3844	pCfB7681
ST7509	Δ*pex10* Atrd11 HsFAR Δ*fao1*	ST3842	pCfB7682
ST7510	Atrd11 HsFAR Δ*hfd1* 100bp_PrGPAT	ST5107	pCfB7681
ST7511	Atrd11 HsFAR Δ*hfd4* 100bp_PrGPAT	ST5110	pCfB7725
ST7514	Δ*pex10* Atrd11 HsFAR 100bp_PrGPAT	ST3842	pCfB5750
ST7787	Atrd11 HsFAR Δ*fao1*	ST3844	pCfB5573

ST7929	Atrd11 HsFAR Δ*fao1* Δ*hfd4*	ST7787	pCfB7869

Table 5: Strains and fatty alcohol production

Strain name	**Strain description**	**Parent strain**	**Plasmids/ BioBricks integrated**	***Z*11-C16:OH [mg/L]**	**Total fatty alcohols [mg/L]**	**Percent *Z*11-C16:OH of total fatty alcohols**
Yarrowia lipolytica GB20	Negative control	N/A	N/A	0 ± 0	0 ± 0	0 ± 0
ST3844	Atrd11 Hs_FAR	*Yarrowia lipolytica* GB20	pCfB3465	0.78 ± 0.07	1.25 ± 0.11	62 ± 2
ST5107	Atrd11 Hs_FAR Δ*hfd1*	ST3844	pCfB5110	2.39 ± 0.5	3.93 ± 0.96	61 ± 5
ST5110	Atrd11 Hs_FAR Δ*hfd4*	ST3844	pCfB5113	1.54 ± 0.70	2.31 ± 0.93	65 ± 6
ST3842	Atrd11 Hs_FAR Δ*pex10*	ST3737	pCfB3516	1.38 ± 0.36	6.04 ± 2.62	24 ± 4
ST5255	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4*	ST5107	pCfB5113	3.46 ± 0.96	7.61 ± 2.13	45 ± 0
ST5452	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10*	ST5450	BB1349/BB1350	4.79 ± 0.47	11.40 ± 0.91	42 ± 4
ST5789	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1*	ST5452	pCfB5573	14.93 ± 3.60	64.46 ± 6.29	23 ± 4
ST5791	Atrd11 Hs_FAR Δ*hfd1* Δ*hfd4* Δ*pex10* Δ*fao1* ΔPrGPAT	ST5789	pCfB5750	26.20 ± 8.85	57.37 ± 19.5	46 ± 1

Example 3: Production of fatty alcohols by yeast

One individual clone of each strain was inoculated into 5 ml YPD medium with 8% glucose (10 g/L yeast extract, 20 g/L peptone, 80 g/L dextrose) in 12-ml glass tubes (Duran, Wertheim, Germany) with metal labocap lids (Lüdiswiss, Flawil, Switzerland) and incubated overnight at 30°C with shaking at 250 rpm. The following day the overnight culture was centrifuged, the supernatant was discarded and the pellet was resuspended in 2 ml nitrogen-limited medium (2.9 g/L (NH4)2SO4, 1.7 g/L YNB (without amino acids and ammonium sulfate), 240 mg/L leucine, 76 mg/L lysine, 20 mg/L uracil and 60 g/L glucose). The cultures were incubated for 48 hours at 30°C and shaken at 250 rpm.

For extraction, 1 mL of culture was transferred into a 4-mL glass vial and 10 µL of internal standard stock (1 µg/µL (Z)-10-heptan-1-yl methyl ester in 100% ethanol) was added. The vials were covered with small pieces of aluminum foil and we used a needle to pierce small holes in the foil covers. The samples were vortexed and placed at -80°C for storage until analysis. The samples were freeze-dried in a freeze dry system (Freezone6 and Stoppening tray dryer, Labconco, Kansas City, USA) at -40°C, then 1 mL chloroform:methanol 2:1 was added to disrupt the cells. The mix was vortexed for 45 s and left at room temperature for 4 hours. The organic solvents were evaporated slowly under a nitrogen stream. 1 ml of hexane was added, the samples were vortexed for 10 s, centrifuged and 200 µl were transferred to a new glass vial. GC-MS analyses were performed on a Hewlett Packard 6890 GC coupled to a mass selective detector HP 5973. The GC was equipped with an INNOWax column (30 m×0.25 mm×0.25 µm), and helium was used as carrier gas (average velocity: 33 cm/s). The MS was operated in electron impact mode (70eV), scanning between m/z 30 and 400, and the injector was configured in splitless mode at 220°C. The oven temperature was set to 80°C for 1 min, then increased at a rate of 10°C /min to 210°C, followed by a hold at 210°C for 15 min, and then increased at a rate of 10 C/min to 230°C followed by a hold at 230°C for 20 min. Compounds were identified by comparison of retention times and mass spectra with those of reference compounds available in laboratory collection. Compounds were quantified by the Total Ion Current (TIC) recorded. Data were analyzed by the Agilent ChemStation software and iWork Numbers. The concentrations of fatty alcohols were calculated based on internal standard (Table 4).

Deletion of genes potentially implicated in fatty alcohol degradation (*HFD1*, *HFD4*, *FAO1*, *PEX10*), improved the production of total fatty alcohols, but decreased the ratio of desaturated alcohol to the total fatty alcohols. Decreasing the expression of the gene encoding for glycerol-3-phosphate acyltransferase (GPAT), which catalyzes the first reaction towards glycerolipids biosynthesis, increased the production of desaturated alcohol, increasing the ratio of desaturated alcohol (Z11-16:OH) to the total alcohols from 23 to 46%. At the same time, the concentration of lipids and free fatty acids (measured as total fatty acid methyl esters) decreased from 1.8 to 0.9 g/L.

Strains from Table 4 were inoculated into 3 mL mineral medium to an optical density (600 nm) of 1 in 24-deep well plates (EnzyScreen, NL). The composition of the mineral medium was as in Jensen et al, 2014, but glucose was replaced by 70 g/L glycerol. The medium was supplemented with 760 mg/L lysine, 760 mg/L leucine and 200 mg/L uracil. The cultures were incubated for 67 hours at 30 ℃ shaken at 250 rpm.
For fatty alcohol extraction, 1 mL of culture was transferred into a 4-mL glass vial and 10 µL of internal standard solution (2 µg/µL (Z)-10-heptan-1-yl methyl ester in 100% ethanol) was added. The vials were covered with small pieces of aluminum foil and a needle was used to pierce small holes in the foil covers. The samples were vortexed and placed at -80℃ for storage until analysis. The samples were freeze-dried in a freeze dry system (Freezone6 and Stoppening tray dryer, Labconco, Kansas City, USA) at -40℃, then 1 mL chloroform:methanol 2:1 was added to disrupt the cells. The mix was vortexed for 45 s and left at room temperature for 4 hours. The organic solvents were evaporated slowly under a nitrogen stream. 1 ml of hexane was added, the samples were vortexed for 10 s, centrifuged and 200 µl were transferred to a new glass vial. Quantification was performed with a SCION TQ GC-MS (Bruker), equipped with an INNOWax 30 m × 0.25 mm × 0.25 µm column, with helium as carrier gas. The injector was configured in splitless mode at 250 ℃, the oven temperature was set to 80 ℃ for 1 min, then increased at a rate of 10 ℃ /min to 210 ℃, followed by a hold at 210 ℃ for 10 min, and then increased at a rate of 10 C/min to 230 ℃ followed by a hold at 230 ℃ for 5 min. The MS was operated in electron impact mode (70eV), scanning between m/z 30 and 350. Compounds were identified by comparison of retention times and mass spectra with those of reference compounds. Compounds were quantified by the Total Ion Current (TIC) recorded. Data were analyzed by the BrukerMSWorkstation software. The concentrations of fatty alcohols were calculated based on internal standards (Figure 6).

All strains expressing the Δ11 desaturase from Δ11 desaturase from *Amyelois transitella* (Atrd11) and the fatty acyl-CoA reductase from *Heliothis subflexa* (HsFAR) produced *(Z)*11-16:OH. When studying the single gene knock-outs, the most prominent effects were observed for FAO1 and PEX10 knock-outs (265 and 406 % improvement in desaturated fatty alcohols). Double deletions were much more effective, .e.g, knocking out both FAO1 and PEX10 gave 877 % increase in desaturated fatty alcohol titer, while the combination of GPAT downregulation and PEX10 knock-out gave a 1083 % increase. Combining several of the successful treatments led to 1475% increase in the titer of desaturated fatty alcohols.

Example 4: Production of fatty alcohols in controlled fermentations

The fermentations were carried out in a BioFlo 415 bioreactor (Eppendorf/New Brunswick), equipped with a 14L vessel (10L max working volume). pH was controlled at 5.0±0.1 with automated addition of a 2N solution of H_2SO_4 and a 4N solution of NaOH. Dissolved oxygen was measured using a polarographic electrode and automatically controlled at 20% saturation by changing the stirring speed of three 6-blade Rushton turbines. Strain *Yarrowia lipolytica* ST6379 was inoculated into the fermentation medium (2 g/L yeast extract, 13.4 g/L yeast nitrogen base, 0.76 g/L lysine, 0.76 g/L uracil, 0.024 mg/L thiamine, 0.002 g/L biotin, and 50 g/L glycerol). After 25 hours of fermentation, the fermentation medium (6 L) was supplemented with 750 ml nutrient-rich feed (which was composed of 16.2 g/L yeast extract, 108.6 g/L yeast nitrogen base, 6.2 g/L lysine, 0.2 mg/L thiamine, 0.02 g/L biotin, and 326 g/L glycerol), followed by a pulse of glycerol to a concentration of 50 g/L in the reactor at 32h. From 36 hours glycerol was fed continuously keeping a steady glycerol concentration of 20-30 g/L.. The fermentation lasted a total of 48 hours. The fatty alcohol concentrations at the end of the fermentation are given in Table 6.

In total 2.86 g/L desaturated fatty alcohols were produced.

Table 6: Production of fatty alcohols by strain ST6379

	Fatty alcohols (g/L)			
Strain	**14:OH**	**16:OH**	**(Z)9-16:OH**	**(Z)11-16:OH**
ST6379	0.116	2.265	0.290	2.570

Example 5: Method to produce Lepidoptera pheromones

Strains ST6527, ST6360 and ST7394 were cultivated in minimal medium as described in Example 3. The medium for strain ST7394 was additionally supplemented with 1 g/L methyl myristate.

By expressing different desaturases and fatty alcohol reductases, the platform yeast strain could be used to produce different desaturared fatty alcohols (Table 7).

Table 7

Strain name	**Strain description**	**Fatty-acyl-CoA desaturase**	**Fatty-acyl-CoA reductase**	**Acetyltrans-ferase**	**Product**
ST6527	Δ*hfd4* Δ*hfd1* Δ*pex10* Δ*fao1*	Atrd11	Har_FAR	none	46.1 ± 1.4 mg/L Z11-hexadecen-1-ol
ST7394	Δ*hfd4* Δ*hfd1* Δ*pex10* Δ*fao1* Δ*hfd2* Δ*hfd3* *GPAT*↓	Dmd9	Har_FAR	Acetyltrans-ferase Atf1 from *S. cerevisiae*	24.9 ± 2.5 mg/L Z9-tetradecen-1-yl acetate
ST6360	Δhfd4 Δhfd1 Δpex10 Δfao1	Atrd11 desaturase from *Spodoptera litura*	Har_FAR Hs_FAR	Acetyltrans-ferase Atf1 from *S. cerevisiae*	27.5 ± 0.9 mg/L Z9-tetradecenanol 15.2 ± 0.8 mg/L Z9-tetradecen-1-yl acetate 25.2 ± 2.0 mg/L Z11-hexadecen-1-ol

Example 6: Method to produce Lepidoptera pheromones

A gene encoding a fatty-acyl-CoA desaturase and a gene encoding a fatty-acyl-CoA reductase are expressed in a *Y. lipolytica* cell with deletions in *PEX10*, *HFD1*, *HFD4* and *FAO1* genes, and with reduced expression of GPAT. The resulting cell, when cultivated in fermentation medium, produces desaturated fatty alcohols. These fatty alcohols are recovered from the broth and chemically oxidised into the corresponding fatty aldehydes.

A gene, encoding a fatty-acyl-CoA desaturase, a gene encoding a fatty-acyl-CoA reductase, are expressed in a *Y. lipolytica* cell overexpressing an acetyltransferase, and having deletions in *PEX10*, *HFD1*, *HFD4* and *FAO1* genes, and with reduced expression of GPAT. The resulting cell, when cultivated in fermentation medium, produces desaturated fatty alcohol acetates.

Table 8. Examples of gene combinations and resulting products.

Fatty-acyl-CoA desaturase	Fatty-acyl-CoA reductase	Acetyltransferase	Product
Δ11 desaturase from *Amyelois transitella*	Reductase from *Helicoverpa armigera*	none	*Z*11-hexadecen-1-ol
Δ9 desaturase from *Drosophila melanogaster* (Fig. 4)	Reductase from *Helicoverpa armigera* (Fig. 4)	Acetyltransferase Atf1 from *S. cerevisiae* (Fig. 5)	*Z*9-tetradecen-1-yl acetate
Δ11 desaturase from *Choristoneura rosaceana*	Reductase from *Helicoverpa armigera*	Acetyltransferase Atf1 from *S. cerevisiae*	Z11-tetradecen-1-yl acetate
Δ11 desaturase from *Choristoneura parallela*	Reductase from *Helicoverpa armigera*	Acetyltransferase Atf1 from *S. cerevisiae*	E11-tetradecen-1-yl acetate

Sequences

SEQ ID NO: 1 – *S. cerevisiae*-codon-optimised nucleotide sequence of *A. transitella* Δ11-desaturase; mRNA-coding sequence.

SEQ ID NO: 2 - Amino acid sequence of *A. transitella* delta-11-desaturase (translation)

SEQ ID NO: 3 Nucleotide sequence of *Y. lipolytica* fatty aldehyde dehydrogenase hfd1 (YALI0F23793g)

SEQ ID NO: 4: Amino acid sequence of *Y. lipolytica* fatty aldehyde dehydrogenase HFD1

SEQ ID NO: 5: Nucleotide sequence of *Y. lipolytica* fatty aldehyde dehydrogenase hfd4 (YALI0B01298g)

SEQ ID NO: 6 Amino acid sequence of *Y. lipolytica* fatty aldehyde dehydrogenase HFD4

SEQ ID NO: 7 Nucleotide sequence of *Y. lipolytica* peroxisome biogenesis factor pex10 (YALI0C01023g) PEX10

SEQ ID NO: 8 Amino acid sequence of *Y. lipolytica* peroxisome biogenesis factor PEX10

SEQ ID NO: 9 Nucleotide sequence of *Y. lipolytica* glycerol-3-phosphate o-acyltransferase gpat (YALI0C00209g)

SEQ ID NO: 10 Amino acid sequence of *Y. lipolytica* glycerol-3-phosphate o-acyltransferase GPAT

SEQ ID NO: 11- *S. cerevisiae*-codon-optimised nucleotide sequence of *H. subflexa* fatty acyl reductase; mRNA-coding sequence.

SEQ ID NO: 12 - Amino acid of *H. subflexa* fatty acyl reductase

SEQ ID NO: 13: HarFAR DNA

SEQ ID NO: 14: HarFAR

SEQ ID NO: 15: HasFAR DNA

SEQ ID NO: 16: HasFAR

SEQ ID NO: 17: Dmed9 DNA

SEQ ID NO: 18: Dmed9

SEQ ID NO: 19: Cro_delta11Z

SEQ ID NO: 20: Cpa_delta11E

SEQ ID NO: 21 FAO1 DNA

SEQ ID NO: 22 Fao1 protein

SEQ ID NO: 23: Ban_FAR DNA

SEQ ID NO: 24: Ban_FAR

SEQ ID NO: 25 PR-141
SEQ ID NO: 26 PR-142
SEQ ID NO: 27 PR-10851 (Atrd11 expression cassette _fw)
SEQ ID NO: 28 PR-10853 (Hs_Far expression cassette _fw)
SEQ ID NO: 29 PR-10655 (EpiVecYL _fw)
SEQ ID NO: 30 PR-10656 (EpiVecYL _rev)
SEQ ID NO: 31 PR-10702 (Δpex10YL_up _fw)
SEQ ID NO: 32 PR-10703 (Δpex10YL_up _rev)
SEQ ID NO: 33 PR-10704 (Δpex10YL_down _fw)
SEQ ID NO: 34 PR-10705 (Δpex10YL_down _rev)
SEQ ID NO: 35 PR-10767 (NatMxSynYL-start _rev_new)
SEQ ID NO: 36 PR-11047 (NatMxSynYL-end _rev_new)
SEQ ID NO: 37 PR-11110 (E.coli backboneUSER _fw)
SEQ ID NO: 38 PR-11111 (E.coli backboneUSER _rev)
SEQ ID NO: 39 PR-11138 (Hphsyn _fw)
SEQ ID NO: 40 PR-11139 (Hphsyn _rev)
SEQ ID NO: 41 PR-11694 (GPAT_up_USER _fw)
SEQ ID NO: 42 PR-11695 (GPAT_down _USER_rev)
SEQ ID NO: 43 PR-13494 (Nat-Tcyc-loxP_fw)
SEQ ID NO: 44 PR-13549 (loxP-PrTefIntron _fw)
SEQ ID NO: 45 PR-14269 (UraYL_fw)
SEQ ID NO: 46 PR-14270 (UraYL_rev)
SEQ ID NO: 47 PR-15426 (Δhfd1_up _fw)
SEQ ID NO: 48 PR-15427 (Δhfd1_up _rev)
SEQ ID NO: 49 PR-15428 (Δhfd1_down _fw)
SEQ ID NO: 50 PR-15429 (Δhfd1_down _rev)
SEQ ID NO: 51 PR-15438 (Δhfd4_up _fw)
SEQ ID NO: 52 PR-15439 (Δhfd4_up _rev)
SEQ ID NO: 53 PR-15440 (Δhfd4_down _fw)
SEQ ID NO: 54 PR-15441 (Δhfd4_down _rev)
SEQ ID NO: 55 PR-16463 (Δfao1YL_up _fw)
SEQ ID NO: 56 PR-16464 (Δfao1YL_up _rev)
SEQ ID NO: 57 PR-16465 (Δfao1YL_down _fw)
SEQ ID NO: 58 PR-16466 (Δfao1YL_down _rev)
SEQ ID NO: 59 PR-16696 (GPAT_up _rev)
SEQ ID NO: 60 PR-16698 (GPAT_100bpPr_down _fw)

SEQ ID NO: 61 *Y.lipolytica* codon-optimized nucleotide sequence of alcohol acetyltransferase from *S. cerevisiae* ATF1

SEQ ID NO: 62 *Saccharomyces cerevisiae* ATF1p amino acid sequence

SEQ ID NO: 63 *Y. lipolytica* codon-optimized nucleotide sequence of Δ9 desaturase from Drosophila melanogaster Dmd9

SEQ ID NO: 64 *Y.lipolytica* codon-optimized nucleotide sequence of fatty acyl reductase from *H. armigera* Har_FAR

SEQ ID NO: 65 *Y. lipolytica* codon-optimized nucleotide sequence of Δ9 desaturase from Spodoptera litura Des11

SEQ ID NO: 66 Δ9 desaturase from *Spodoptera litura* Des11

SEQ ID NO: 67 *Y. lipolytica* codon-optimized nucleotide sequence of Δ11 desaturase from *Amyelois transitella*

SEQ ID NO: 68 Amino acid sequence of Δ11 desaturase from *Amyelois transitella*

SEQ ID NO: 69 *Y. lipolytica* codon-optimized nucleotide sequence of fatty acyl reductase from *Helicoverpa armigera*

SEQ ID NO: 70 Amino acid sequence of fatty acyl reductase from *Helicoverpa armigera*

SEQ ID NO: 71	PR-10714
SEQ ID NO: 72	PR-10766
SEQ ID NO: 73	PR-10767
SEQ ID NO: 74	PR-11047
SEQ ID NO: 75	PR-13338
SEQ ID NO: 76	PR-141
SEQ ID NO: 77	PR-14148
SEQ ID NO: 78	PR-14149
SEQ ID NO: 79	PR-142
SEQ ID NO: 80	PR-14565
SEQ ID NO: 81	PR-14566
SEQ ID NO: 82	PR-14567
SEQ ID NO: 83	PR-14568
SEQ ID NO: 84	PR-14589
SEQ ID NO: 85	PR-14590
SEQ ID NO: 86	PR-14591
SEQ ID NO: 87	PR-14592
SEQ ID NO: 88	PR-15430
SEQ ID NO: 89	PR-15431

SEQ ID NO: 90	PR-15432
SEQ ID NO: 91	PR-15433
SEQ ID NO: 92	PR-15434
SEQ ID NO: 93	PR-15435
SEQ ID NO: 94	PR-15436
SEQ ID NO: 95	PR-15437
SEQ ID NO: 96	PR-15930
SEQ ID NO: 97	PR-16592
SEQ ID NO: 98	PR-16593
SEQ ID NO: 99	PR-16594
SEQ ID NO: 100	PR-16595
SEQ ID NO: 101	PR-14149
SEQ ID NO: 102	PR-14279
SEQ ID NO: 103	PR-14395
SEQ ID NO: 104	PR-14396
SEQ ID NO: 105	PR-14397
SEQ ID NO: 106	PR-14398
SEQ ID NO: 107	PR-15521
SEQ ID NO: 108	PR-15522
SEQ ID NO: 109	PR-18486
SEQ ID NO: 110	PR-18487
SEQ ID NO: 111	PR-18490
SEQ ID NO: 112	PR-18489
SEQ ID NO: 113	PR-18499
SEQ ID NO: 114	PR-18500

References

Alfaro, Navarro-Llopis, Primo, 2009. Optimization of pheromone dispenser density for managing the rice striped stem borer, *Chilo suppressalis* (Walker), by mating disruption. Crop Protection. 28:567-572.

Angerer, Radermacher, Mankowska, Steger, Zwicker, Heide, Wittig, Brandt, Zickermann, 2014. The LYR protein subunit NB4M/NDUFA6 of mitochondrial complex I anchors an acyl carrier protein and is essential for catalytic activity. PNAS. 111(14)

Bari, 2003. Development of pheromone mating disruption strategies for the suppression of the artichoke plume moth in artichokes grown on the central coast of

California. ISHS Acta Horticulturae 660: V International Congress on Artichoke. doi: 10.17660/ActaHortic.2004.660.80

Chen, Beckerich, Gaillardin, 1997. One-step transformation of the dimorphic yeast Yarrowia lipolytica. Appl Microbiol Biotechnol. 48(2):232-5

Eizaguirre, Sans, López, Albajes. 2002. Effects of mating disruption against the Mediterranean corn borer, Sesamia nonagrioides, on the European corn borer Ostrinia nubilalis. Use of pheromones and other semiochemicals in integrated production IOBC wprs Bulletin.

Ferrell, Yao, 1972. Reductive and oxidative synthesis of saturated and unsaturated fatty aldehydes, J Lipid Res. 13(1):23-6.).

Iwama R, Kobayashi S, Ohta A, Horiuchi H, Fukuda R. 2014; 289(48):33275-86. J Biol Chem. Fatty aldehyde dehydrogenase multigene family involved in the assimilation of n-alkanes in Yarrowia lipolytica.

Jensen, Strucko, Kildegaard, David, Maury, Mortensen, Forster, Nielsen, Borodina, 2014. EasyClone: method for iterative chromosomal integration of multiple genes in *Saccharomyces cerevisiae*, FEMS Yeast Res. 14(2):238-48

Iwama R, Kobayashi S, Ohta A, Horiuchi H, Fukuda R. FEMS Yeast Res. 2015 May;15(3). Alcohol dehydrogenases and an alcohol oxidase involved in the assimilation of exogenous fatty alcohols in *Yarrowia lipolytica*.

Kehat, Dunkelblum, 1993. Sex Pheromones: achievements in monitoring and mating disruption of cotton pests in Israel, Achieves of Insect Biochemistry and Physiology. 22:425-431.

Li, Zhang, 2009. An environmentally benign TEMPO-catalyzed efficient alcohol oxidation system with a recyclable hypervalent iodine(III) reagent andIts facile preparation. Synthesis, 1163-1169a.

Meyer, Schreiber, 1994. Acceleration of the Dess-Martin oxidation by water J. Org. Chem., 59, 7549-7552;

Okada, Asawa, Sugiyama, Kirihara, Iwai, Kimura, 2014. Sodium hypochlorite pentahydrate (NaOCl·5H2O) crystals as an extraordinary oxidant for primary and secondary alcohols. Synlett, 25, 596-598.

Steves J.E. and Stahl S.S., 2013. Copper(I)/ABNO-catalyzed aerobic alcohol oxidation: alleviating steric and electronic constraints of Cu/TEMPO catalyst systems. J. Am. Chem. Soc., 135, 15742-15745

Stovicek V, Borja GM, Forster J, Borodina I. EasyClone 2.0: expanded toolkit of integrative vectors for stable gene expression in industrial Saccharomyces cerevisiae strains. J Ind Microbiol Biotechnol. 2015 Nov;42(11):1519-31.

Sumita T, Iida T, Hirata A, Horiuchi H, Takagi M, Ohta A. (2002) FEMS Microbiol Lett. 2002 Aug 27;214(1):31-8. "Peroxisome deficiency represses the expression of n-alkane-inducible YlALK1 encoding cytochrome P450ALK1 in *Yarrowia lipolytica*."

Tamura, Aoyama, Takido, Kodomari, 2012. Novel [4-Hydroxy-TEMPO + NaCl]/SiO2 as a reusable catalyst for aerobic oxidation of alcohols to carbonyls. Synlett, 23, 1397-1407.

Yadav, Reddy, Basak, Narsaiah, 2004. Recyclable 2nd generation ionic liquids as green solvents for the oxidation of alcohols with hypervalent iodine reagents, Tetrahedron, 60, 2131-2135.

Wu, Zhang, Yao, Xu, Wang and Zhang, 2012. Management of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) by mating disruption. Insect Science 19 (6), 643–648.

Items

1. An oleaginous yeast cell capable of producing a desaturated fatty alcohol, said cell:
   i) expressing at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and
   ii) expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
   iii) having reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology,

such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

2. The yeast cell according to item 1, wherein the yeast cell has reduced activity of Pex10 (SEQ ID NO: 8) and of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), or reduced activity of Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

3. The yeast cell according to item 2, wherein the yeast is of the *Yarrowia* species, preferably the yeast is *Yarrowia lipolytica*.

4. The yeast cell according to any one of the preceding items, wherein the at least one heterologous desaturase is selected from the group consisting of a Δ3 desaturase, a Δ5 desaturase, a Δ6 desaturase, a Δ7 desaturase, a Δ8 desaturase, a Δ9 desaturase, a Δ10 desaturase, a Δ11 desaturase, a Δ12 desaturase, a Δ13 desaturase and a Δ14 desaturase.

5. The yeast cell according to any one of the preceding items, wherein the reduction in activity of Hfd1, Hfd4, Pex10 and Fao1 or a protein having at least 60% homology thereto is partial or total, and/or wherein the reduction in activity of GPAT or a protein having at least 60% homology thereto is partial.

6. The yeast cell according to any one of the preceding items, wherein the desaturase is derived from an organism of an insect, such as from the *Lepidoptera* order.

7. The yeast cell according to any one of the preceding items, wherein the desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2.

8. The yeast cell according to any one of the preceding items, wherein the desaturase is a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18.

9. The yeast cell according to any one of the preceding items, wherein the desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 19.

10. The yeast cell according to any one of the preceding items, wherein the desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 20.

11. The yeast cell according to any one of the preceding items, wherein the FAR is selected from:
    i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14;
    ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16;
    iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and
    iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24.

12. The yeast cell according to any one of the preceding items, further expressing an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate.

13. The yeast cell according to any one of the preceding items, wherein at least one of the genes encoding a desaturase, a fatty acyl-CoA reductase or an acetyltransferase is present in high copy number.

14. The yeast cell according to any one of the preceding items, wherein at least one of the genes encoding a desaturase, a fatty acyl-CoA reductase or an acetyltransferase is under the control of an inducible promoter.

15. The yeast cell according to any one of the preceding items, wherein at least one of the genes encoding a desaturase, a fatty acyl-CoA reductase or an acetyltransferase is codon-optimised for said yeast cell.

16. The yeast cell according to any one of the preceding items, wherein the genes encoding a desaturase, a fatty acyl-CoA reductase or an acetyltransferase are comprised within the genome of the cell or within a vector comprised within the cell.

17. The yeast cell according to any one of the preceding items, wherein the cell comprises a deletion or a mutation of at least one of *HFD1* (SEQ ID NO: 3), *HFD4* (SEQ ID NO: 5), *PEX10* (SEQ ID NO: 7), *FAO1* (SEQ ID NO: 21) and/or a mutation of *GPAT* (SEQ ID NO: 9), or a deletion or a mutation in at least one nucleic acid sequence having at least 60% homology thereto, such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology thereto, resulting in reduction of activity of at least one of Hfd1, Hfd4, Pex10, Fao1 and GPAT.

18. The yeast cell according to item 17, comprising a mutation of in *PEX10* (SEQ ID NO: 7) and at least one of *HFD1* (SEQ ID NO: 3), *HFD4* (SEQ ID NO: 5), *FAO1* (SEQ ID NO: 21) and/or *GPAT* (SEQ ID NO: 9) or a homologue thereof.

19. The yeast cell according to any one of the preceding items, wherein *HFD1* (SEQ ID NO: 3), *HFD4* (SEQ ID NO: 5), *PEX10* (SEQ ID NO: 7) and/or *FAO1* (SEQ ID NO: 21) or a homologue thereof having at least 60% homology thereto is deleted or mutated, resulting in total loss of activity of Hfd1, Hfd4, Pex10 and/or Fao1, and/or wherein *GPAT* (SEQ ID NO: 9) or a homologue thereof having at least 60% homology thereto is mutated, resulting in reduced activity of GPAT.

20. The yeast cell according to any one of the preceding items, wherein the cell has reduced or total loss of activity of at least two of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

21. The yeast cell according to any one of the preceding items, wherein the cell has reduced or total loss of activity of at least three of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

22. The yeast cell according to any one of the preceding items, wherein the cell has reduced or total loss of activity of four of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

23. The yeast cell according to any one of the preceding items, wherein the cell has reduced or total loss of activity of all of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

24. The yeast cell according to any one of the preceding items, wherein the fatty acyl-CoA reductase is encoded by a nucleic acid sequence identical to or having at least 90% homology to SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least

95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology, such as 100% homology to SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15.

25. The yeast cell according to any one of the preceding items, wherein the cell is capable of producing fatty alcohols with a total titre of at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L, wherein the total titre is the sum of the titre of desaturated fatty alcohols and the titre of saturated fatty alcohols.

26. The yeast cell according to any one of the preceding items, wherein the desaturated fatty alcohols produced by the cell represent at least 20% of the total fatty alcohols produced by the cell, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90% of the total fatty alcohols produced by the cell.

27. The yeast cell according to any one of the preceding items, wherein the cell is capable of producing (*Z*)11-C16:OH with a titre of at least 0.5 mg/L, such as at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L (*Z*)11-C16:OH.

28. The yeast cell according to any one of the preceding items, wherein the (*Z*)11-C16:OH produced by the cell represents at least 20% of the total fatty alcohols produced by the cell, such as at least 30%, such as at least 40%, such as at least 50%, such as at least 60%, such as at least 70%, such as at least 80%, such as at least 90% of the total fatty alcohols produced by the cell.

29. A method of producing a desaturated fatty alcohol and/or a desaturated fatty acyl acetate in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or reduced activity of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

30. The method according to item 27, wherein the yeast cell has a mutation resulting in reduced activity of Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or has a mutation resulting in reduced activity of at least one protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity or at least one one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), , Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such

as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

31. The method according to any one of items 29 to 30, wherein the yeast cell is as defined in any one of items 1 to 29.

32. The method according to any one of items 29 to 31, further comprising the step of converting the desaturated fatty alcohol of step ii) into a fatty acyl acetate or a fatty aldehyde.

33. The method according to any one of items 29 to 32, further comprising the step of recovering the desaturated fatty alcohol, the fatty acyl acetate and/or the aldehyde.

34. The method according to any one of items 29 to 33, further comprising the step of formulating the desaturated fatty alcohol, the fatty acyl acetate and/or the aldehyde in a pheromone composition.

35. The method according to any one of items 29 to 34, wherein the pheromone composition further comprises one or more additional compounds such as a liquid or solid carrier or substrate.

36. The method according to any one of items 29 to 33, wherein at least part of the desaturated fatty alcohol is Z11-16:OH.

37. A nucleic acid construct for modifying a yeast cell, said construct comprising:

i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) optionally, additional polynucleotides for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10),

wherein optionally the first polynucleotide and/or the second polynucleotide and/or the additional polynucleotides are under the control of a promoter.

38. The nucleic acid construct according to item 37, further comprising a third polynucleotide encoding at least one acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate.

39. A kit of parts comprising:

a) the yeast cell according to any one of items 1 to 28 and instructions for use; and/or

b) a nucleic acid construct according to any one of items 37 or 38, wherein said construct is for modifying a yeast cell, and

c) optionally the yeast cell to be modified.

40. The kit of parts according to item 39, wherein the yeast cell to be modified has reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

41. A desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the method according to any one of items 29 to 36.

42. Use of a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the method according to any one of items 29 to 36.

Claims

1. An oleaginous yeast cell capable of producing a desaturated fatty alcohol, said cell:

i) expressing at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) expressing at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) having a mutation resulting in reduced activity of Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having a mutation resulting in reduced activity of at least one protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

2. The yeast cell according to claim 1, wherein the yeast is of the *Yarrowia* species, preferably the yeast is *Yarrowia lipolytica*.

3. The yeast cell according to any one of the preceding claims, wherein the at least one heterologous desaturase is selected from the group consisting of a Δ3 desaturase, a Δ5 desaturase, a Δ6 desaturase, a Δ7 desaturase, a Δ8

desaturase, a Δ9 desaturase, a Δ10 desaturase, a Δ11 desaturase, a Δ12 desaturase, a Δ13 desaturase and a Δ14 desaturase.

4. The yeast cell according to any one of the preceding claims, wherein the reduction in activity of Hfd1, Hfd4, Pex10 and Fao1 or a protein having at least 60% homology thereto is partial or total, and/or wherein the reduction in activity of GPAT or a protein having at least 60% homology thereto is partial.

5. The yeast cell according to any one of the preceding claims, wherein the desaturase is derived from an organism of an insect, such as from the *Lepidoptera* order, preferably the desaturase is a Δ11 desaturase having at least 60% homology to the Δ11 desaturase from *Amyelois transitella* as set forth in SEQ ID NO: 2 or a Δ9 desaturase having at least 60% homology to the Δ9 desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 18,.

6. The yeast cell according to any one of the preceding claims, wherein the FAR is selected from:

i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 14;

ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 16;

iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 12; and

iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 24,

preferably the FAR has at least 80% homology to the FAR from *Helicoverpa armigera* or to the FAR from *Heliothis subflexa*.

7. The yeast cell according to any one of the preceding claims, further expressing an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate.

8. The yeast cell according to any one of the preceding claims, wherein the cell is capable of producing fatty alcohols with a total titre of at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at

least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L, wherein the total titre is the sum of the titre of desaturated fatty alcohols and the titre of saturated fatty alcohols.

9. The yeast cell according to any one of the preceding claims, wherein the cell is capable of producing (*Z*)11-C16:OH with a titre of at least 0.5 mg/L, such as at least 1 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, such as at least 6 g/L, such as at least 7 g/L, such as at least 8 g/L, such as at least 9 g/L, such as at least 10 g/L (*Z*)11-C16:OH.

10. A method of producing a desaturated fatty alcohol and/or a desaturated fatty acyl acetate in an oleaginous yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell:

i) expresses at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

ii) expresses at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and

iii) has a mutation resulting in reduced activity of Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or having a mutation resulting in reduced activity of at least one protein having at least 60% homology to Pex10 (SEQ ID NO: 8) and a mutation resulting in reduced activity or at least one one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), , Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10), such as at least 65% homology, such as at least 70% homology, such as at least 75% homology, such as at least 80% homology, such as at least 81% homology, such as at least 82% homology, such as at least 83% homology, such as at least 84% homology, such as at least 85% homology, such as at

least 86% homology, such as at least 87% homology, such as at least 88% homology, such as at least 89% homology, such as at least 90% homology, such as at least 91% homology, such as at least 92% homology, such as at least 93% homology, such as at least 94% homology, such as at least 95% homology, such as at least 96% homology, such as at least 97% homology, such as at least 98% homology, such as at least 99% homology to Pex10 (SEQ ID NO: 8) and at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10).

11. The method according to claim 10, wherein the yeast cell is as defined in any one of claims 1 to 9.

12. A nucleic acid construct for modifying a yeast cell, said construct comprising:

i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA; and

ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and

iii) additional polynucleotides for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or of at least one protein having at least 60% homology to one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Pex10 (SEQ ID NO: 8), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10); preferably for reducing activity of at least Pex10 (SEQ ID NO: 8) or a protein having at least 60% homology thereto and optionally for reducing activity of at least one of Hfd1 (SEQ ID NO: 4), Hfd4 (SEQ ID NO: 6), Fao1 (SEQ ID NO: 22) and GPAT (SEQ ID NO: 10) or a protein having at least 60% homology thereto;

wherein optionally the first polynucleotide and/or the second polynucleotide and/or the additional polynucleotides are under the control of a promoter.

13. A kit of parts comprising:

a) the yeast cell according to any one of claims 1 to 9 and instructions for use; and/or

b) a nucleic acid construct according to claim 12, wherein said construct is for modifying a yeast cell, and

c) optionally the yeast cell to be modified.

14. A desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the method according to any one of claims 10 to 11.

15. Use of a desaturated fatty alcohol, a desaturated fatty acyl acetate or a desaturated fatty aldehyde obtainable by the method according to any one of claims 10 to 11.

FIG. 1

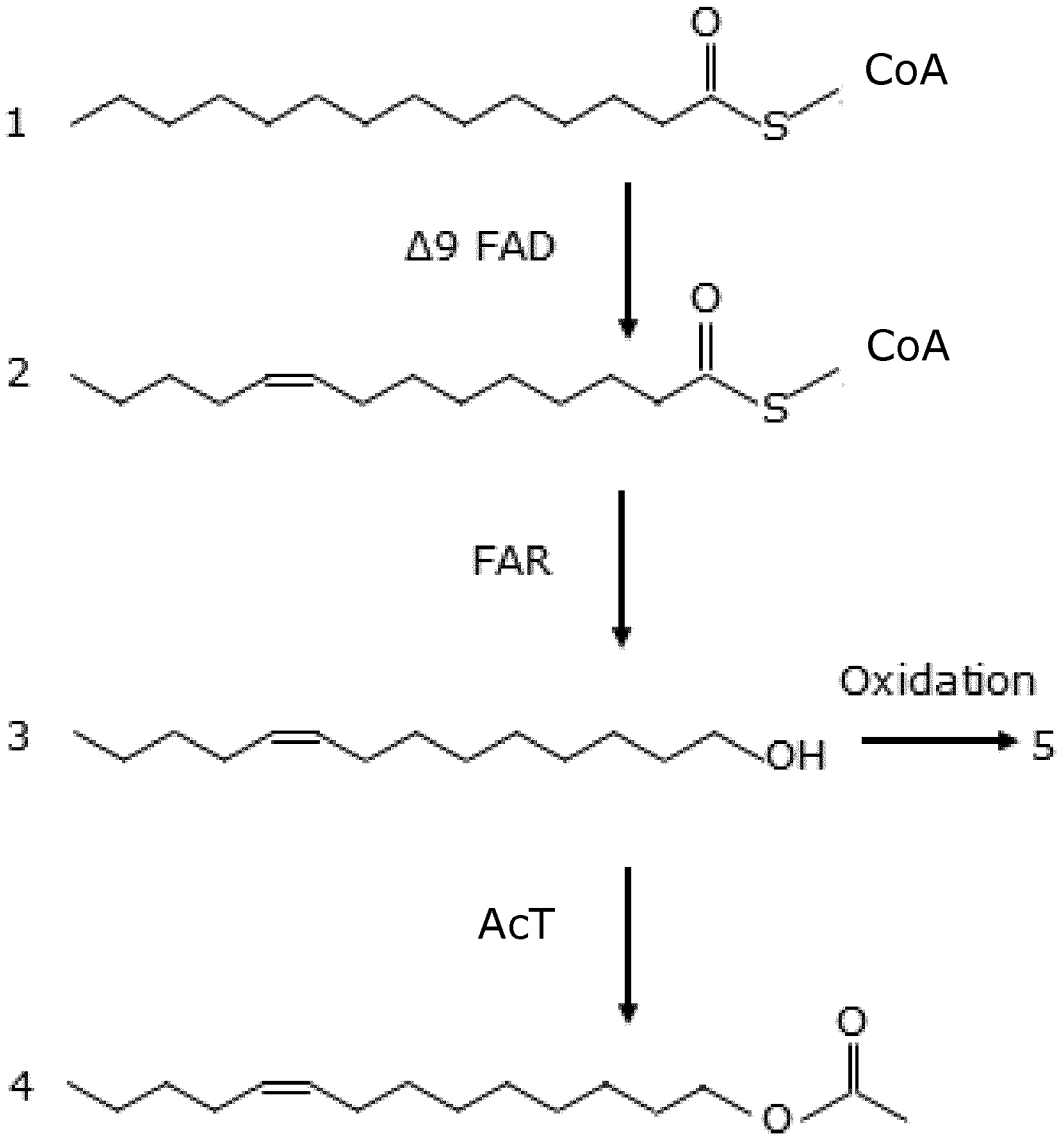


FIG. 2

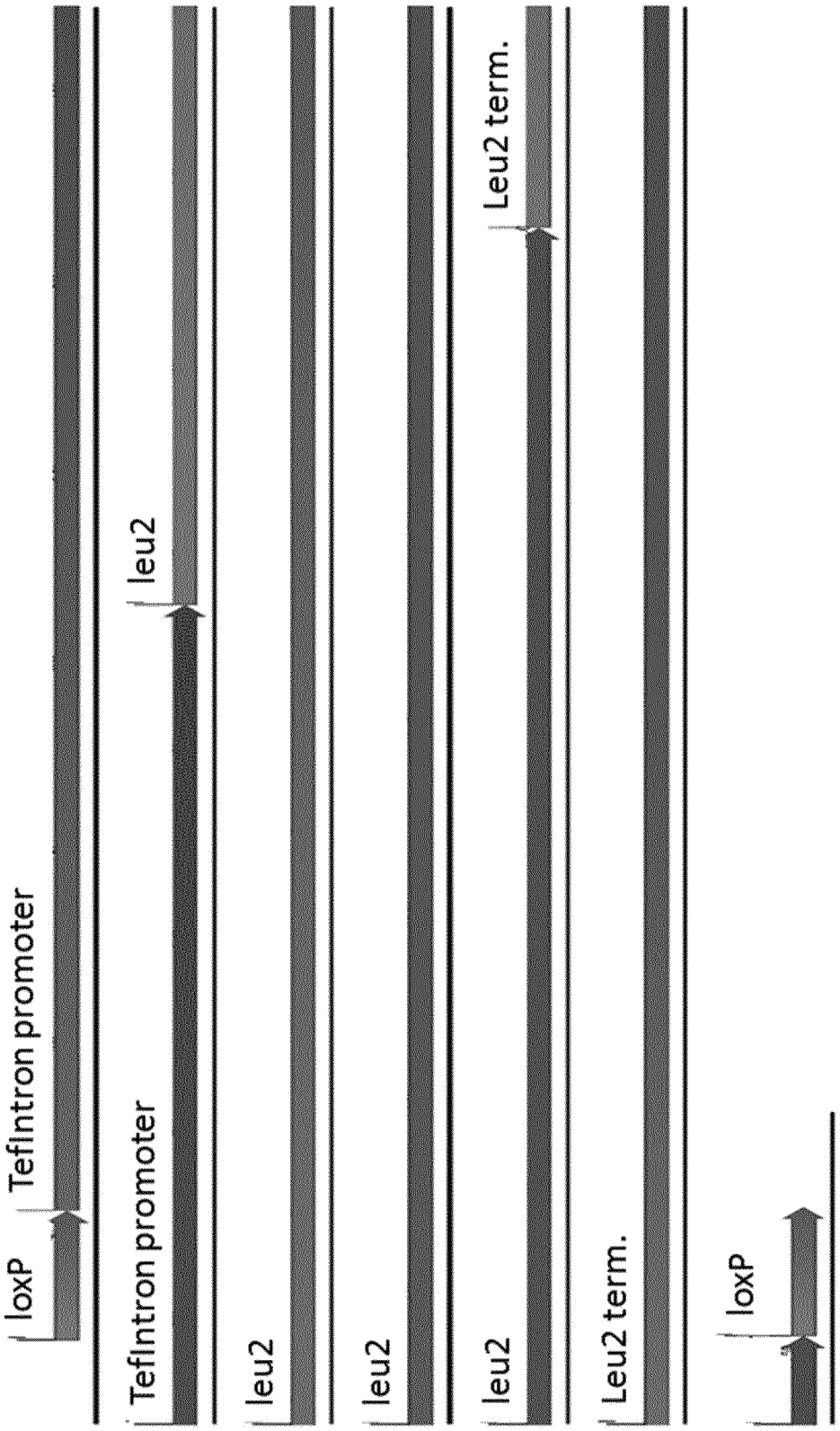

loxP
TefIntron promoter
TefIntron promoter
leu2
leu2
leu2
leu2
Leu2 term.
Leu2 term.
loxP

FIG. 3

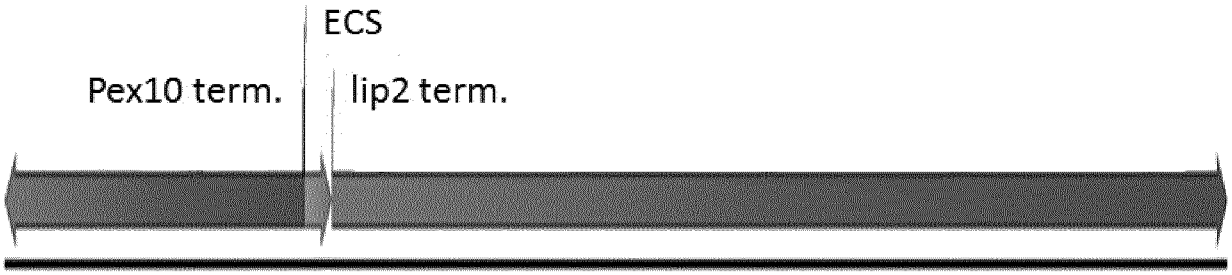


FIG. 4

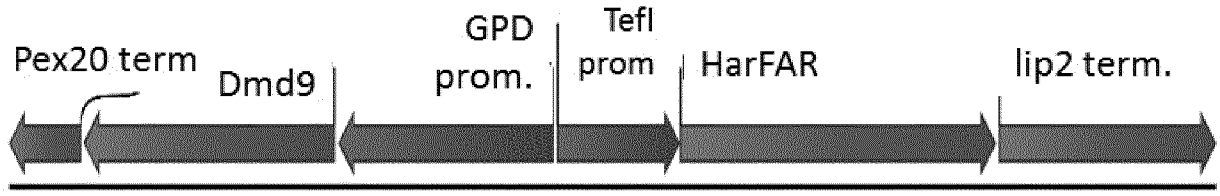


FIG. 5

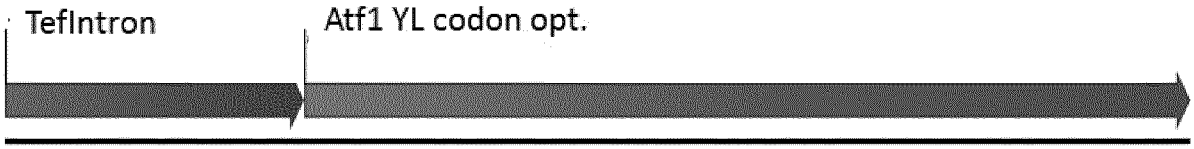
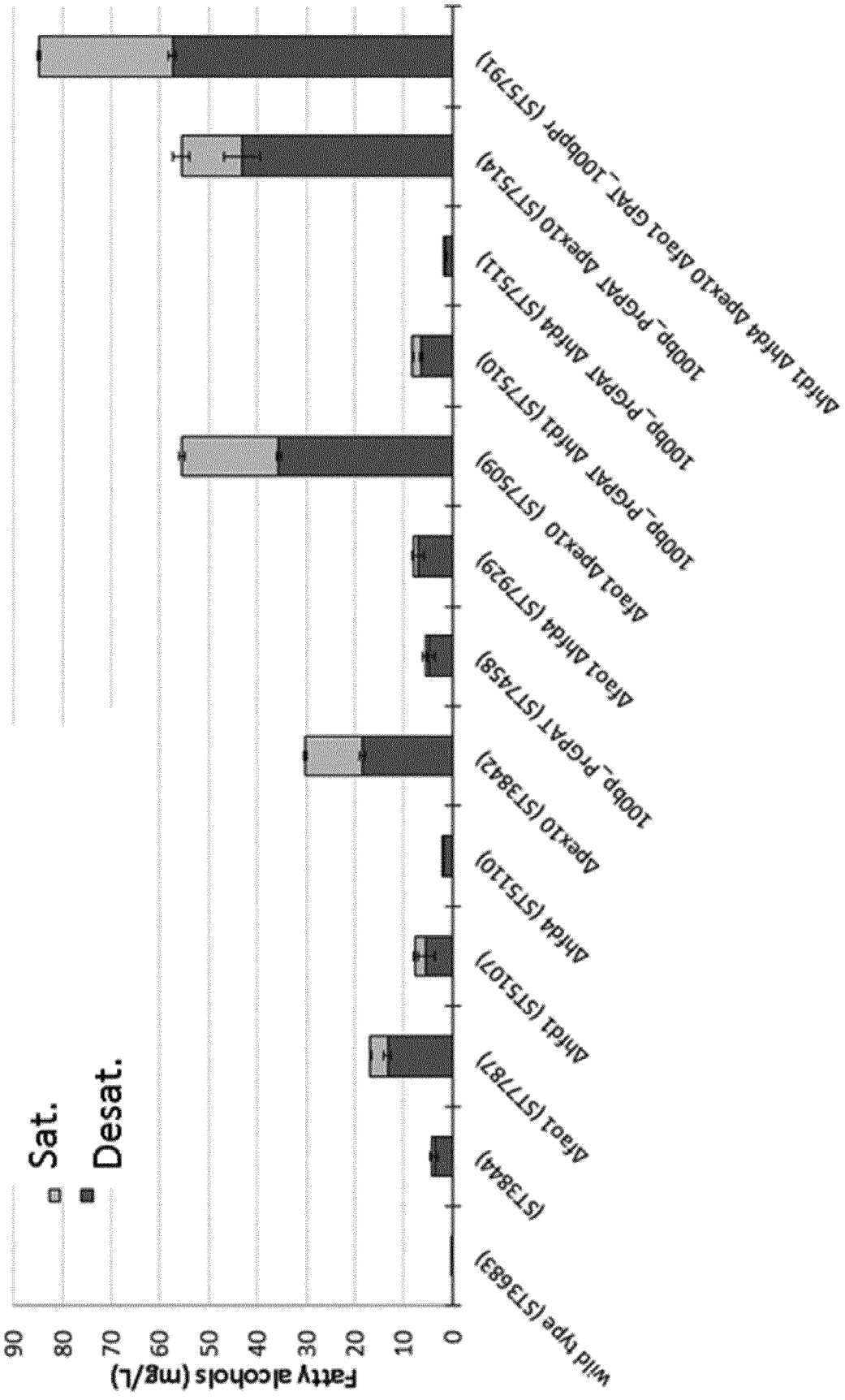


FIG. 6


Sat.
Desat.
Fatty alcohols (mg/L)
0
10
20
30
40
50
60
70
80
90
wild type (ST6683)
(ST3844)
Afao1 (ST7787)
Ahfd1 (ST5107)
Ahfd4 (ST5110)
Apex10 (ST3842)
100bp_PrGPAT (ST7458)
Afao1 Ahfd4 (ST7929)
Afao1 Apex10 (ST7509)
100bp_PrGPAT Ahfd1 (ST7510)
100bp_PrGPAT Ahfd4 (ST7511)
100bp_PrGPAT Apex10 (ST7514)
Ahfd1 Ahfd4 Apex10 Afao1 GPAT_100bpPr (ST7791)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083021

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/02 C12P7/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ÅSA K HAGSTRÖM ET AL: "A moth pheromone brewery: production of (Z)-11-hexadecenol by heterologous co-expression of two biosynthetic genes from a noctuid moth in a yeast cell factory", MICROBIAL CELL FACTORIES, BIOMED CENTRAL, GB, vol. 12, no. 1, 13 December 2013 (2013-12-13), page 125, XP021172484, ISSN: 1475-2859, DOI: 10.1186/1475-2859-12-125	14,15
Y	abstract	1-13
	----- -/--	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2018	08/03/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUOKUN WANG ET AL: "Exploring fatty alcohol-producing capability of Yarrowia lipolytica", BIOTECHNOLOGY FOR BIOFUELS, vol. 9, no. 1, 20 May 2016 (2016-05-20), XP055379246, DOI: 10.1186/s13068-016-0512-3 abstract figures 1,3,5 page 3 - page 4, column 1, paragraph 2 -----	1-13
Y	DING BAO-JIAN ET AL: "The Yeast ATF1 Acetyltransferase Efficiently Acetylates Insect Pheromone Alcohols: Implications for the Biological Production of Moth Pheromones", LIPIDS, SPRINGER, DE, vol. 51, no. 4, 22 January 2016 (2016-01-22), pages 469-475, XP035947504, ISSN: 0024-4201, DOI: 10.1007/S11745-016-4122-4 [retrieved on 2016-01-22] abstract page 470, column 1, paragraph 3 - column 2, paragraph 2 -----	7

1